SYNTHESIS AND EVALUATION OF *IN-VITRO* HEPATOPROTECTIVE ACTIVITY OF NOVEL 1,2,3,4- TETRAHYDRO QUINAZOLINE DERIVATIVES

Dissertation submitted to

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In partial fulfillment for the award of the Degree of

MASTER OF PHARMACY

IN

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Submitted by

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APRIL – 2014

CERTIFICATE

This is to certify that the dissertation entitled "**SYNTHESIS AND EVALUATION OF *IN-VITRO* HEPATOPROTECTIVE ACTIVITY OF NOVEL 1,2,3,4- TETRAHYDRO QUINAZOLINE DERIVATIVES ",** is a bonafide work done by **Mrs.R.NIRMALA [Reg. No. 261215202],** J.K.K. Nattraja College of Pharmacy, in partial fulfillment of the University rules and regulations for award of Master of Pharmacy in Pharmaceutial Chemsitry under my guidance and supervision during the academic year 2013-14.

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This is to certify that the work embodied in this dissertation entitled "**SYNTHESIS AND EVALUATION OF *IN-VITRO* HEPATOPROTECTIVE ACTIVITY OF NOVEL 1,2,3,4- TETRAHYDRO QUINAZOLINE DERIVATIVES ",** submitted to "The Tamil Nadu Dr. M.G.R. Medical University", Chennai, in partial fulfillment to the requirement for the award of Degree of Master of Pharmacy in Pharmaceutical Chemistry, is a bonafide work carried out by **Mrs.R.NIRMALA [Reg.No.261215202]**, during the academic year 2013-2014 under the guidance and supervision of **Dr. M.VIJAYABASAKAN, M.Pharm.,Ph.D.,** Head Department of Pharmaceutical Chemistry , J.K.K. Nattraja College of Pharmacy, Kumarapalayam.

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EVALUATION CERTIFICATE

This is to certify that the work embodied in this dissertation entitled "**SYNTHESIS AND EVALUATION OF *IN-VITRO* HEPATOPROTECTIVE ACTIVITY OF NOVEL 1,2,3,4- TETRAHYDRO QUINAZOLINE DERIVATIVES"** submitted to "The Tamil Nadu Dr. M.G.R. Medical University", Chennai, in partial fulfillment to the requirement for the award of Degree of Master of Pharmacy in Pharmaceutical Chemistry, is a bonafide work carried out by **Mrs.R.NIRMALAR [Reg.No.261215202]**, during the academic year 2013-2014, was evaluated by us during the examination held on ……..

Internal Examiner **External Examiner**

DECLARATION

I hereby declare that the dissertation entitled **"SYNTHESIS AND EVALUATION OF *IN-VITRO HEPATOPROTECTIVE ACTIVITY OF NOVEL 1,2,3,4-TETRAHYDRO QUINAZOLINE DERIVATIVES''*,** is based on the original work carried out by me under the guidance of **Dr.M.Vijayabaskaran M.Pharm.,Ph.D.,** Professor and Head Department of Pharmaceutical Chemistry for the submission to The Tamilnadu Dr. M. G. R. Medical University, Chennai in partial fulfillment of the requirement for the award of Degree of **MASTER OF PHARMACY** in Pharmaceutical Chemistry during the academic year 2013-14.

The work is original and has not been submitted in part or any Degree of this or any other University. The information furnished in this dissertation is genuine to the best of my knowledge and Belief.

PLACE: Kumarapalayam **Mrs. R. NIRMALA**

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1. INTRODUCTION

The subject of medicinal chemistry explains the design and production of compounds that can be used for the prevention, treatment or cure of human and animal diseases. Medicinal chemistry includes the study of already existing drugs, of their biological properties and their structure-activity relationships [1]. Medicinal chemistry was defined by IUPAC specified commission as it concerns the discovery, the development, the identification and the interpretation of the mode of action of biologically active compounds at the molecular level.

1.1.Medicinal chemistry covers the following stages

- In the first stage new active substances or drugs are identified and prepared from natural sources, organic chemical reactions or biotechnological processes. They are known as lead molecules.
- The second stage is optimization of lead structure to improve potency, selectivity and to reduce toxicity.
- Third stage is development stage, which involves optimization of synthetic route for bulk production and modification of pharmacokinetic and pharmaceutical properties of active substance to render it clinically useful.

Medicinal chemistry is the application of chemical research techniques to the synthesis of pharmaceuticals. During the early stages of medicinal chemistry development, scientists were primarily concerned with the isolation of medicinal agents found in plants. Today, scientists in this field are also equally concerned with the creation of new synthetic compounds as drugs. Medicinal chemistry is almost always geared toward drug discovery and development [2].

Medicinal chemists apply their chemistry training to the process of synthesizing new pharmaceuticals. They also work on improving the process by which other pharmaceuticals are made. Most chemists work with a team of scientists from different disciplines, including biologists, toxicologists, pharmacologists, theoretical chemists, microbiologists, and biopharmacists. Together this team uses sophisticated analytical techniques to synthesize and test new drug products and to develop the most cost-effective and eco-friendly means of production. Man is slightly nearer to the atom than the stars. From his central position he can survey the grandest works of Nature with the astronomer or the minutest with the chemist.

Heterocyclic chemistry is the chemistry branch dealing exclusively with synthesis, properties and application of heterocyclic. A Heterocyclic compound is defiend as any organic compound where their molecules are characterized by rings containing at least one atom other than carbon and hydrogen. Such as sulfur, oxygen or nitrogen as part of the ring. The word “hetero” means “ different from carbon and hydrogen ’’. Many hetero cyclic compounds are biosynthesized by plants and animals and they are biologically active. Some hetero cyclic compounds are fundamentals of life like, haeme derivatives in blood and chlorophyll is essential for photosynthesis in plants. Several hetero cycles are the basic structure nucleus for nicotine ,pyridoxine, cocaine, morphine etc., They may be either simple aromatic ring or non-aromatic rings. Some heterocyclic compounds are known as carcinogens. Researchers have show that heterocyclic amines are the carcinogenic chemicals.

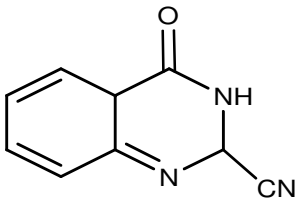
Among the family of heterocyclic compounds, nitrogen containing heterocyclic especially Quinazolines show a number of biological activities: antimicrobial, anti-inflammatory, analgesic, anticonvulsant, antiulcer, anticancer, anti tubercular activity, antihypertensive [3].

1.2. QUINAZOLINE

Quinazoline is a compound made up of two fused six member simple aromatic rings-benzene and pyrimidine ring. Medicinally it is used as antimalarial agent. It was first prepared by Gabriel in 1903 and first isolated from the Chinese plant aseru. The development of research on biological activity of quinazoline compounds started when the compound 2-methyl-1,3-aryl-4-quinazoline derivative was synthesized. This compound has soporific & sedative action. In last 10 to 15 years of research for medicinal has been characterized by significant advances.

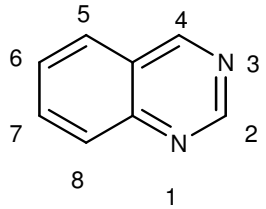
In 1968 only two derivatives were used methaqualone and diuretic quinathazone. In 1980 about 50 kinds of the quinazolines were derived with various kinds of disease.

In 1869 Griess prepared the first quinazoline derivative, 2–cyano-3,4-dihydro-4-oxoquinazoline, by the reaction of cyanogens with anthranilic acid. Griess apparently recognized the bicylic nature of the product which he called bicyanoamido benzoyl and used this name until 1885.



The preparation of the parent quinazoline came many years later when Bischler and Lang obtained it by decarboxylation of the 2-carboxy derivative.

A more satisfactory synthesis of quinazoline was subsequently devised by Gabriel in 1903 that studied properties and those of its derivatives in greater detail [4].



The name was proposed by Widdege. Other names such as phenmiazine, benzyleneamidine, benzo-1, 3-diazine, 5, 6-benzopyrimidine and 1, 3-diazanapthaline have occasionally been used. The numbering suggested by Paal and Busch is still in use. The presence of a fused benzene ring alters the properties of the pyrimidine ring considerably. The two nitrogen atoms are not equivalent, and the marked polarization of the 3, 4- double bond is reflected in the reactions of quinazoline [5]. The properties of substitute's quinazolines depend largely on

- The nature of the substitutents.
- Whether they are in the pyrimidine ring (or) in the benzene ring.
- Whether (or) not complete conjugation is present in the pyrimidine ring.

The chemistry of quinazoline was reviewed by Williamson in 1957, then by Lindquist in 1959 and brought up to date by Armarego in 1963.

1.2.1. Chemical Properties

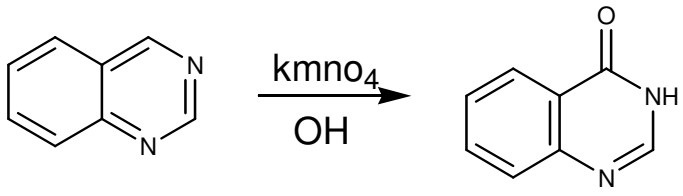
Quinazoline is stable in cold, dilute acid and alkaline solutions, but it is destroyed when these solutions are boiled. O-Aminobenzaldehyde, ammonia and formic acid are formed when quinazoline is boiled with hydrochloric acid.

1.2.2. **Hydrolysis, oxidation and reduction**

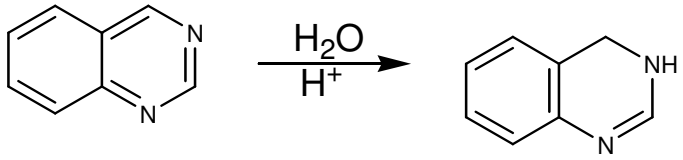
Oxidation of quinazoline in dilute aqueous acid, with two equivalents of hydrogen peroxide at room temperature gave a high yield of 3, 4- dihydro-4-oxo quinazoline.

In alkaline medium, where the anhydrous neutral species of quinazoline were predominantly undergoing oxidation with $KMnO_4$ furnished a high yield of 3, 4-dihydro-4-oxo quinazoline was also formed [6].

Oxidation



Reduction



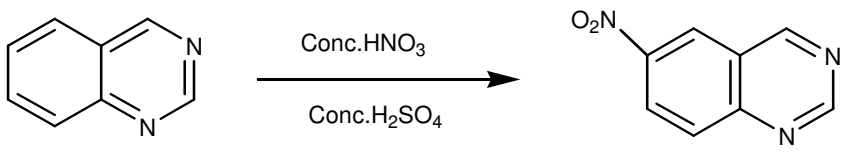
Catalytic hydrogenation of quinazoline stopped after the absorption of one molecules of hydrogen and gave 3, 4-dihydro quinazoline.

Reduction with sodium amalgam gave 1,2,3,4-tetrahydroquinazoline. Lithium aluminum hydride and sodium borohydride gave 3,4-dihydro and 1,2,3,4-tetrahydroquinazoline.

1.2.3. Nucleophilic and electrophilic substitution reactions

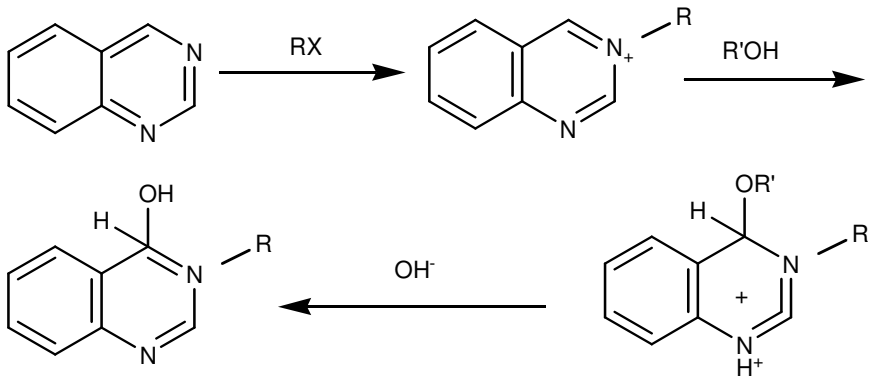
The two known nucleophilic substitution reactions of quinazoline namely with sodamide and hydrazine, presumably proceed via the intermediate addition products and gave 4-amino and 4-hydrazine quinazoline.

Nitration is the only known electrophilic substitution reaction of quinazoline. Theoretical considerations show that the expected order of reactivity is at positions 8 > 6 > 5 > 7 > 4 > 2. Quinazoline gives 6-nitroquinazoline with fuming nitric acid in concentrated H_2SO_4. No oxidation of the heterocyclic ring can occur under these conditions because the hydrated cation is not present [7].



1.2..4. Alkylation reactions

Alkylation of quinazoline takes place on N, 3-methyl, 3-ethyl-3-alkyl and 3-benzyl quinazolinium salts readily take up a molecule of alcohol to form the corresponding 4-alkoxy-3-alkyl-3,4-dihydro quinazolinium salts. These salts yield the pseudo bases, 3-alkyl-3, 4-dihydro-4-hydroxy quinazolines on treatment with strong alkali.



1.2.5. Addition Reactions

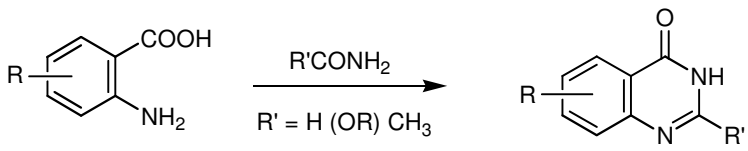
Quinazoline is very reactive towards anionid reagents which attack position 4. Sodium bisulphate, hydrogencyanide, acetophenone, acetone, 2- butanone and cyclohexanone add across the 3,4-double bond of quinazoline. Methyl, ethyl, isopropyl, benzyl, t-butyl and phenyl magnesium halides and phenyl lithium also add across the 3, 4-double bond to give the corresponding 4-substituted 3, 4-dihydroquinazolines [8]

1.2.6. Synthesis

Following methods were reported for the synthesis of oxoquinazolines**.**

Niementowski's Synthesis

Niementowski's found that 3 (or) 4 substituted anthranilic acid when reacted with formamide at 125 - 130ºC for 4 hours gave 86% yield of 3, 4-dihydro-4-oxoquinazoline

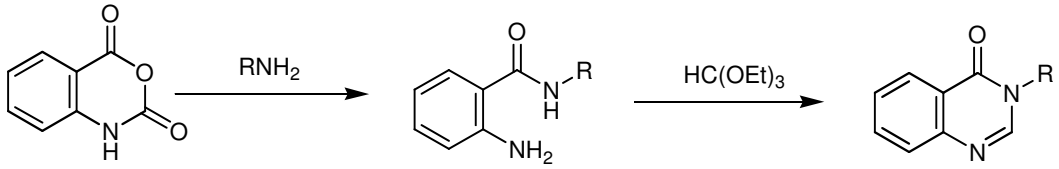


Grimmel, Guinther and Morgan's synthesis

3 moles of O-amino benzoic acids, when heated with 3 moles of an amine together with one mole of phosphorous trichloride in toluene for two hours, gave high yields of 2, 3-disubstituted 3,4-dihydro-4-oxoquinazoline

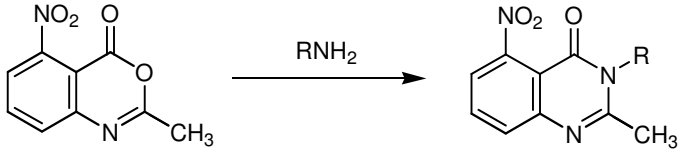
From Isatoic Anhydride

Isatoic anhydride readily reacts with equimolar quantity of amines to dihydro-4-oxoquinazolines by refluxing ethyl orthoformate for 1- 6hours without isolating the intermediate amides.



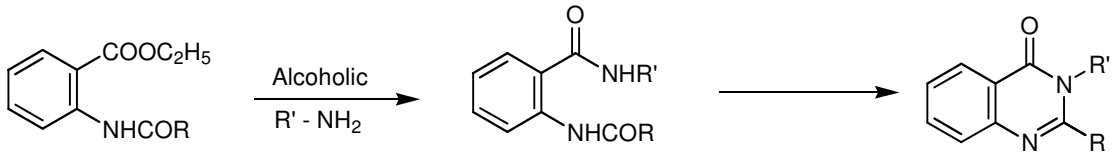
From 3, 1, 4-Benoxazones (Acylanthranils) and amines.

3, 1, 4-Benoxazones react with amines to give 3, 4-dihydro-4-oxoquinazolines. Primary aliphatic amines and anilines react with 2-methyl-5-nitro-4-oxoquinazolines.

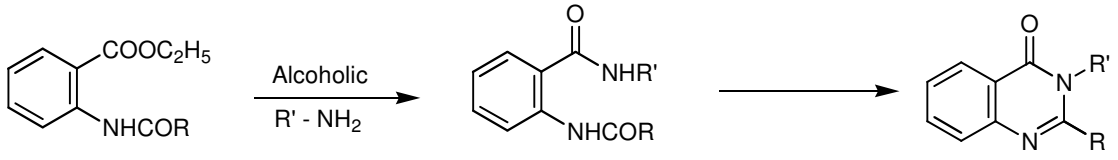


From ethyl 2-acetamido-5-nitrobenzoate

Ethyl 2-acetamido-5-nitrobenzene and alcoholic ammonia when heated in a sealed tube at 170oC yields 3,4-dihydro-methyl-6-nitro4-oxoquinazoline.

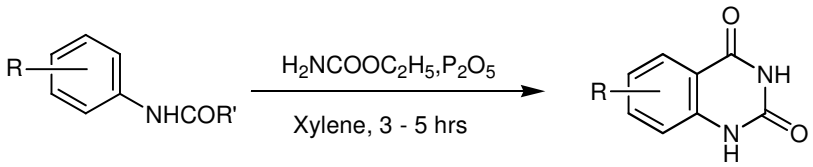


Ethyl 2-acetamido-5-nitrobenzene and alcoholic ammonia when heated in a sealed tube at 170ºC, yields 3,4-dihydro –methyl-6-nitro-4-oxoquinazoline.



Sen and Ray's synthesis

Boiling a solution of normal (or) isobutyrylanilides with urethane and phosphorous pentoxide in xylene gave 2-propyl and 2-isopropyl-3, 4-dihydro-4-oxoquinazolines.

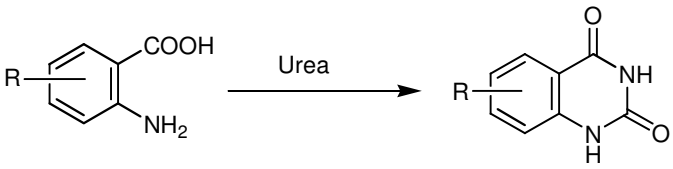


(R = Me, OMe, OEt; R' = Me, Et, Pr, Iso-Pro, Ph)

Following methods were reported for the synthesis of 2,4-dioxoquinazolines.

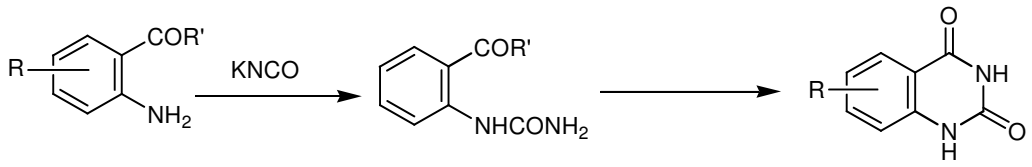
From anthranilic acid and urea

The fusion of anthranilic acid with urea to give 1,2,3,4-tetrahydro-2,4-dioxoquinazoline was first described by Griess.



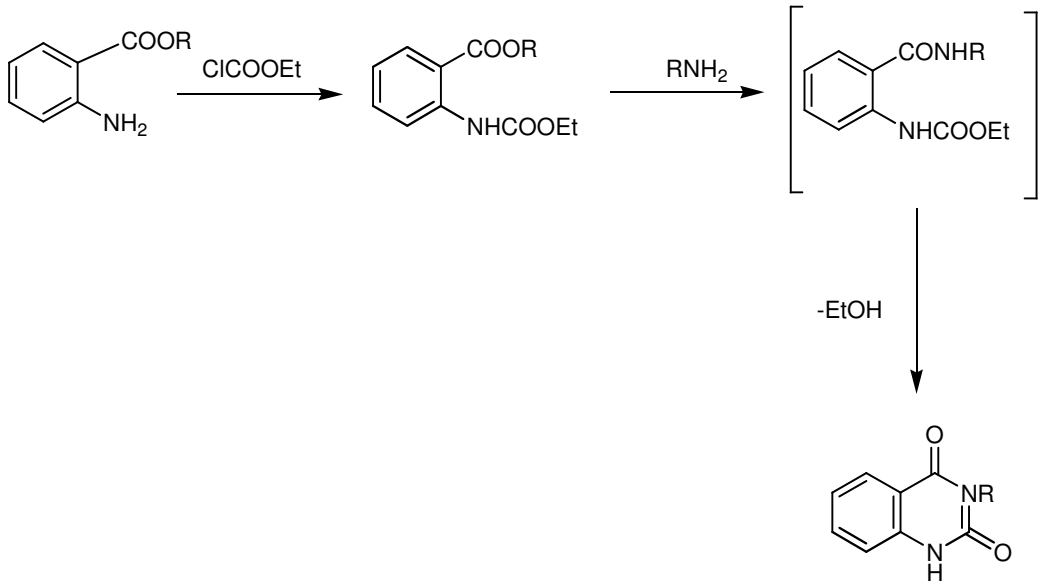
From O-ureidobenzoic acid

O-ureidobenzoic acids are readily prepared from the corresponding anthranilic acid and potassium cyanate. The ureido acids are then easily cyclised to the respective 1,2,3,4-tetrahydro-2,4-dioxoquinazolines by heating with acid (or) alkali. Anthranilic esters and amides as well as undergo this reaction.



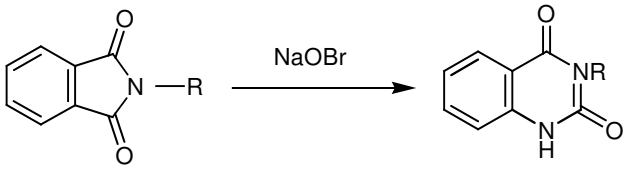
From O- ethoxy carbonyl amino benzoic esters (or) amides

When O-ethoxy carbonyl amino benzamide and its 4-methyl derivatives are heated above their melting points, they lose water and from 1, 2, 3, 4-tetrahydro-2, 4-dioxoquinazoline



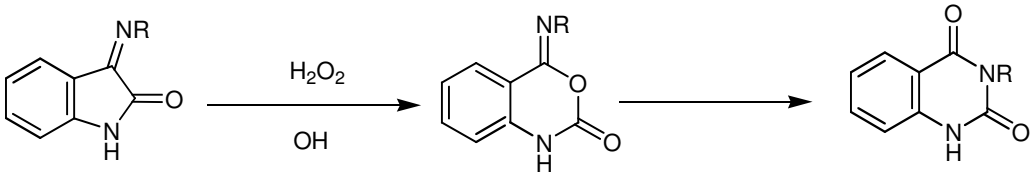
From phthalic acid derivatives

The use of derivatives of phthalic acid for the preparation of dioxoquinazoline necessitates rearrangement of the Hoffmann curties (or) Lossan type. Reaction of phthalamide (or) phthalimide, N-methyl and N-ethyl phthalimide with alkali hypobromite gives the 1, 2, 3, 4-tetrehydro 2, 4-dioxoquinazoline.s



From Isatins

α-isatin oxime rearranges to 1,2,3,4-tetrahydro-2,4-dioxoquinazoline on heating with dilute sodium hydroxide, β-imino derivatives of isatin, on the other hand, require oxidation with hydrogen peroxide in alkaline solution in order to from the dioxoquinazoline.



1.3. MOLECULAR DOCKING

In the field of molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using for example scoring functions.

Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs.

1.3.1. Autodock

Auto dock 4 is a suite of automated docking tools. It is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3d structure.

1.3.2. Auto dock 4 comprises three major improvements.

- The docking results are more accurate and reliable.
- It can optionally model flexibility in the target macromolecule.
- It enables Auto dock's use in evaluating protein – protein interactions.

1.3.3. Applications

A binding interaction between a small molecule ligand and a enzyme protein may result in activation or inhibition of the enzyme. If the protein is a receptor, ligand binding may results in agonism or antagonism. Docking is most commonly used in the field of drug design – most drugs are small organic molecules, and docking may be applied to:

- **Hit identification** – docking combined with a scoring function can be used to quickly screen large databases of potential drugs *in silico* to identify molecules that likely to bind to protein target of interest.
- **Lead optimization** – docking can be used to predict in where and in which relative orientation a ligand binds to protein. This information may in turn be used to design more potent and selective analogs [9].

1.4.DRUG LIKENESS

Drug likeness is a qualitative concept used in drug design for how "druglike" a substance is with respect to factors like bioavailability. It is estimated from the molecular structure before the substance is even synthesized and tested. A druglike molecule has properties like this

- Optimal solubility to both water and fat, because an orally administered drug has to go through the intestinal lining, carried in aqueous blood and penetrate the lipid cellular membrane to reach the inside of a cell. The model compound for the cellular membrane is octanol the logarithm of the octanol/water partition coefficient, known as ClogP, is used to estimate solubility.
- Since the drug is transported in aqueous media like blood and intracellular fluid, it has to be sufficiently water-soluble. Solubility in water can be estimated from the number of hydrogen bond donors vs. alkyl side chains in the molecule. Low water solubility translates to slow absorption and action. Too many hydrogen bond donors, on the other

hand, lead to low fat solubility, so that the drug cannot penetrate the cell wall to reach the inside of the cell.

- **Molecular weight:** the smaller, the better, because diffusion is directly affected. 80% of traded drugs have molecular weights under 450 daltons; they belong to the group ofsmall molecules. Substructures that have known pharmacological properties. Also, other factors such as substructures with known toxic, mutagenic or teratogenic properties affect the usefulness of a designed molecule. In fact, several poisons have a good drug likeness. Natural toxins are used in pharmacological research to find out their mechanism of action, and if it could be exploited for beneficial purposes.
- Drug likeness indexes are inherently limited tools. Drug likeness can be estimated for any molecule, and does not evaluate the actual specific effect that the drug achieves (biological activity) .Furthermore, first-pass metabolism which is biochemically selective, can destroy the pharmacological activity of a compound despite good drug likeness. Drug likeliness was calculated based on “Lipinski’s rule of five". The score is computed using many commercial and academic softwares like Molinspiration etc [10].

1.4.1. Lipinski's Rule of Five [11]

Lipinski's Rule of Five is a rule of thumb to evaluate druglikeness or determine if a chemical compound with a certain pharmological or biological activity has properties that would make it a likely orally active drug in humans. The rule was formulated by Christopher A.Lipinski in 1997, based on the observation that most medication drugs are relatively small and lipophillic molecules.

The rule describes molecular properties important for a drug's pharmacokinetics in the human body, including their absorption,distribution,metabolism, and excretion("ADME"). However, the rule does not predict if a compound is pharmacologically active.

The rule is important for drug development where a pharmacologically active lead structureis optimized step-wise for increased activity and selectivity, as well as drug-like properties as described by Lipinski's rule. The modification of the molecular structure often leads to drugs with higher molecular weight, more rings, more rotatable bonds and a higher lipophilicity.

1.5.HEPATIC DISEASES

Liver disease is any disturbance of liver function that causes illness. The liver is responsible for many critical functions within the body and should it become diseased or injured, the loss of those functions can cause significant damage to the body. Liver disease is also referred to as hepatic disease. Liver disease is a broad term that covers all the potential problems that may occur to cause the liver to fail to perform its designated functions. Usually, more than 75% or three quarters of liver tissue needs to be affected before decrease in function occurs

The liver the largest solid organ in the body; and is also considered a gland because among its many functions, it makes and secretes bile. The liver is located in the upper right portion of the abdomen protected by the rib cage. It has two main lobes that are made up of tiny lobules. The liver cells have two different sources of blood supply. The hepatic artery supplies oxygen rich blood that is pumped from the heart, while the portal vein supplies nutrients from the intestine and the spleen.

Normally, veins return blood from the body to the heart, but the portal vein allows chemicals from the digestive tract to enter the liver for "detoxification" and filtering prior to entering the general circulation. The portal vein also efficiently delivers the chemicals and proteins that liver cells need to produce the proteins, cholesterol, and glycogen required for normal body activitiess

1.5.1. Infectious hepatitis

The term "hepatitis" means inflammation, and liver cells can become inflamed because of infection.

Hepatitis A is a viral infection that is caused primarily through the fecal-oral route when small amounts of infected fecal matter are inadvertently ingested. Hepatitis A causes an acute inflammation of the liver which generally resolves spontaneously. The hepatitis vaccine can prevent this infection.

Hepatitis B is spread by exposure to body fluids (needles from drug abusers, contaminated blood, and sexual contact) and can cause an acute infection, but can also progress to cause chronic inflammation (chronic hepatitis) that can lead to cirrhosis and liver cancer. The hepatitis B vaccine prevents this infection.

Hepatitis C causes chronic hepatitis. An infected individual may not recall any acute illness. Hepatitis C is spread by exposure to body fluids (needles from drug abusers, contaminated blood, and sexual contact). Chronic hepatitis C may lead to cirrhosis and liver cancer. At present, there is no vaccine against this virus.

Hepatitis D is a virus that requires concomitant infection with hepatitis B to survive, and is spread via body fluid exposure (needles from drug abusers, contaminated blood, and sexual contact).

Hepatitis E is a virus that is spread via contaminated food and water exposure.

1.5.2. Treatment for liver disease

Each liver disease will have its own specific treatment regimen. For example, hepatitis A requires supportive care to maintain hydration while the body's immune system fights and resolves the infection. Patients with gallstones may require surgery to remove the gallbladder. Other diseases may need long-term medical care to control and minimize the consequences of their disease

In patients with cirrhosis and end-stage liver disease, medications may be required to control the amount of protein absorbed in the diet. The liver affected by cirrhosis may not be able to metabolize the waste products, resulting in elevated blood ammonia levels and hepatic

encephalopathy. Low sodium diet and water pills (diuretics) may be required to minimize water retention.

In those with large amounts of ascites fluid, the excess fluid may have to be occasionally removed with a needle and syringe (paracentesis). Using local anesthetic, a needle is inserted through the abdominal wall and the fluid withdrawn [12].

Some Marketed available quinazoline derivatives.

1.Prazosin

Chemical Name : 2-[4-furoyl) piperazine-1-yl]-6,7-dimethoxy quinazolin-4-amine

Use : Sympatholytic , Treatment of high Blood pressor.

Another Name : Minipress , vasoflex , pressin and Hypovase.

2.Gefitinib

Chemical Name : N-(3-chloro-4-fluoro-phenyl)-7-methoxy-6-(3-morpholin-4-yl-propyl) quinazolin-4-amine

Use : In the treatment of certain type of cancer , EGFR inhibitor

Another Name : Astra Zeneca

3.Erlotinib

Chemical Name : N-(3-ethnyl phenyl)-6,7-bis(2-methoxy ethoxy) quinazolin-4-amine

Use : In the Treatment of Pancreatic cancer, Lung cancer inhibitor of EGFR , thyrosine kinase inhibitor.

4.Tetrodotoxin (TTX)

Chemical Name : (4R , 4aR , 5R , 6S , 7S , 8S , 8aR , 10S , 12S)-2-azaiumylidene-4,6,7,12-tetrahydroxy-6-(hydroxyl methyl)-2,3,4,4a,5,6,7,8-octahydro-1H-8a,10-methano-5,7-epoxy methanoxy)quinazolin-10-olate.

Use : Potent neurotoxin

5. Alfuzosin

Chemical Name : N-[3-[(4-amino-6,7-dimethoxy-quinazolin-2-yl)-methyl-amino]propyl]- tetrahydra furan-2-carboxamide.

Use : alpha-1 receptor antagonist , Benign Hyper Prostatic Hyperplasia(BPH)

2. OBJECTIVE AND PLAN OF WORK

The synthesis of Tetrahydroquinazoline and their derivatives has been of considerable interest to organic and medicinal chemists for many years as indicated by large number of drugs. Tetrahydroquinazoline show a number of biological activities: like,

(1) Antimicrobial (2) Anticonvulsant (3) Anti-inflammatory etc.

The molecular manipulation of promising lead compound is still a major line of approach for the discovery of new drugs. Molecular manipulation involves the efforts to combining the separate groups having similar activity in one compound. Thus by making gradual changes in the physico-chemical properties of the drug and thus the biological activity of the compound. Combination of two or more moieties into one is a common procedure of manipulation and this can probably result in the increase of biological activity and removal of untoward side effects. It is my effort in the present work to synthesize tetrahydroquinazoline derivatives by using different aldehydes.

2.1. Steps involved in plan of work

Synthesis and Characterization

- Synthesis of title compounds.
- Study of physico-chemical properties of the synthesized compounds.
- Spectral Studies.
  The chemical structure of the synthesized compounds were characterized by means of IR, ^{1}H-NMR, Mass spectral study.

2.2. Biological screening

To evaluate the *in vitro* hepatoprotective activity of the title compounds.

3. REVIEW OF LITERATURE

1. **Won-Jea C *et al.,*** have studied the virtual screening was employed for hit compound identification with chemical libraries using Surflex Dock. Subsequently, hit optimization for potent and selective candidate JAK2 inhibitors was performed through synthesis of diverse C-1 substituted quinazoline derivatives [13].
2. **Rajveer CH *et al.,*** have been synthesized a number of substituted oxoquinazolines & reported their analgesic & anti-bacterial activity [14].
3. **Vouy LT and Michelle M *et al.,*** has done the Condensation of o-iodobenzaldehydes with amidine hydrochlorides under ligand-free copper catalyzed Ullmann N-arylation conditions afforded the corresponding quinazolines in good to excellent yield [15].
4. **Patil JP *et al.,*** reported the synthesis of 2-methyl-3(H)-Quinazolinone by microwave irradiation method [16].
5. **Florea Dumitrascu *et al.,*** have been synthesized pyrrol (1,2-c)quinazoline and investigated using X-ray diffraction and their NOE experiments in high resolution NMR [17].
6. **Deepti Kohli et al.,** have been synthesized quinazolinone derivatives and evaluated for their antibacterial activity by cup plate method by measuring inhibition zone [18].
7. **Talersa GL et al**., have been treated the benzoxazine 1 with hydrazine hydrate in ethanol furnished 3 amino-2-phenylquinazolin-4-(3H)-one 2, which upon condensation with aldehydes yielded the corresponding 3-arylidenoamino derivatives. Cyclization of these derivatives using mercaptosuccinic acid afforded 1, 3-thiazolidin-4-one ethanolic acids, which after esterfication with N-hydroxyphthalimide or N-hydroxysuccinamide via acid chlorides produced the respective ethanolic esters [19].
8. **Salahuddin MD et al.,** have been synthesized a series of novel 3-(6-substituted-1, 3-benzothiazole-2-yl)-2-[{(4-substituted phenyl) amino} methyl] quinazoline-4(3*H*)-ones and the synthesized quinazoline-4-one derivatives were investigated for their

anti-inflammatory and antibacterial activity [20].

9. **Olayinka O. Ajani et al.,** have been synthesized a series of novel quinoxalin-2(1*H*)-one-3-hydrazone derivatives, 2a - 8d were synthesized via condensation of 3 hydrazinoquinoxalin-2(1*H*)-one, with the corresponding ketones under microwave irradiation. The microwave assisted reaction was remarkably successful and gave hydrazones in higher yield at less reaction time compared to conventional heating method [21].

10. **Chandrasekara Reddy G *et al.,*** have done the docking studies of few newly synthesized 6,7-dialkoxy-4-anilinoquinazoline derivatives which showed EGFR-TK inhibitory [22].

11. **Gazi Irez *et al.,*** have been synthesized (hydroxyimino) (2-phenyl(1,2,3,4 tetrahydroquinazolin-2-yl))methane and (hydroxyimino)(2-(2-thienyl)(1,2,3,4-tetrahydroquinazolin-2-yl))methane were synthesized by the condensation of 2-(hydroxyimino)-1-phenylethan-1-one and 2-(hydroxyimino)-1-(2-thienyl)ethan-1-one with 2-aminobenzylamine (2-ABA). Complexes of these ligands with Co3+were prepared with a metal: ligand ratio of 1:2. The ligands and their complexes were elucidated on the basis of elemental analyses, AAS, FT-IR, 1H- and ^{13}C-NMR spectra, mass spectra, magnetic susceptibility measurements, molar conductivity [23].

12. **Varsha Jatav *et al.*,** have been synthesized 3-[5-(4- substituted phenyl)-1, 3, 4-thiadiazole-2-yl]-2-styryl quinazoline -4(3H)-ones and reported their antibacterial and antifungal activity [24].

13. **Varsa Jatav *et al.,*** have been synthesized 3-[5-substituted-1, 3, 4-thiadiazole-2-yl]-2-styryl quinazoline-4(3H)-ones and their CNS depressant activity was screened with the help of forced swim pool method [25].

14. **Yuliang Wang *et al.,*** have designed and synthesized series of 4-(2-methoxyphenyl)-2-oxobutylquinazoline derivatives and reported their anticoccidial activity [26].

15. **Praveen Kumar P *et al.,*** have been synthesized 6,7,8,9-tetrahydro-5(H)-5-nitrophenylthiazolo[2,3-b]-quinazolin-3(2H)-one derivatives and the synthesized compounds have been screened for antimicrobial activity [27].

16. **Omar Abd el-Fattah M. Fathalla *et al.,*** have been synthesized the new series of some 2-[(E)-2-furan-2-yl-vinyl]-quina- zolin-4(3H)-ones incorporated into pyrazoline, isoxazoline, pyrimidine or pyrimidine-thione ring systems at position-3 of the quinazoline ring. The antimicrobial activity and anti-inflammatory effect of some of these compounds were studied [28].

17. **Omar Al-Deeb *et al.,*** have been synthesized a series of 21 new 2-alkylthio-6-iodo-3-substituted-quinazolin-4-one derivatives was prepared and screened for their in vitro antitubercular activity against *Mycobacterium tuberculosis* strain HRv, using the radiometric BACTEC 460-TB methodology [29].

18. **Siddappa K *et al.*,** have been synthesized 3-[(2-Hydroxy-quinolin-3-ylmethylene)-amino]-2-phenyl- 3H-quinazolin-4-one and its Metal (II) Complexes and reported their antimicrobial activity [30].

19. **Cedric Loge *et al.,*** have done Continuous efforts in microwave-assisted synthesis and the structure activity relationships' (SARs) studies of novel modified 9-oxo-thiazolo[5,4-f]quinazoline-2-carbonitriles, allowed identification of new amidine and imidate derivatives as potent and dual CDK1/GSK-3 inhibitors. Combination of lead optimization and molecular modeling studies allowed identification of a dual CDK1/GSK-3 inhibitor (compound 13d) with submicromolar values [31].

20. **Gloria D Galarce *et al.*,** This study describes the effect of novel 6-Arylbenzimidazo [1,2-*c*]quinazoline derivatives as tumor necrosis factor alpha (TNF-α) production inhibitors. The newly synthesized compounds were tested for their *in* vitroability to inhibit the lipolysaccharide (LPS) induced TNF- α secretion in the human promyelocytic cell line HL-60. The compound 6-Phenyl-benzimidazo [1,2-*c*]quinazoline, coded as G1, resulted as the most potent inhibitor and with no significant cytotoxic activity. Thus, 6-Arylbenzimidazo [1,2-*c*]quinazoline derivatives may have a potential as anti-inflammatory agents [32].

21. **Suthakaran R *et al.,*** have been synthesized by condensing 2-methyl / phenyl / Chloro methyl disubstituted benzooxazine-4-one and 1-(2- amino ethyl)– 4- substituted benzylidene-2-phenyl-1H–Imidazoles–5(4H)-one, gave 30 imidazolo quinazoline-4- one derivatives. All the compounds have been screened for their antimicrobial activities. Most of the compounds have shown promising antibacterial, and antifungal activity [33].

22. **Chan Seong Cheong *et al*.,** have been Studied on the selective reduction of 1*H*-Quinazoline-2,4-diones [34].

23. **Ashraf A. Aly *et al.*,** have been synthesized a series of triazoloquinazolinones and benzimidazoquinazolinones has been achieved under microwave irradiation. The products were obtained in nearly quantitative yield within few minutes during the reaction of aromatic aldehydes with 5-amino-1(*H*)-1,2,4-triazole (or 2-aminobenzimidazole) and dimedone in DMF [35].

24. **Periyasamy Selvam *et al.,*** have designed and synthesized novel 2,3-disubtituted quinazoline-4(3H)-ones by microwave technique and characterized them by spectral analysis & synthesized compounds were screened for cytotoxicity and for antiviral activity against influenza A [36].

25. **Veerachamy *et al*.**, have been synthesized a series of 3-benzyl-2-substituted-3H-[1,2,4]triazolo[5,1-b]quinazolin-9-ones have been synthesized by the cyclo condensation of 3-amino-2-benzylamino-3H-quinazolin-4-one with a variety of one-carbon donors. The compounds were evaluated for their in vivo antihypertensive activity using spontaneously hypertensive rats (SHR). While all the test compounds exhibited significant antihypertensive activity,3-benzyl-2-methyl-3H-[1,2,4]triazolo[5,1-b]quinazolin-9-one exhibited antihypertensive activity more than the reference standard prazocin [37].

26. **Desai N C *et al*.,** have been reported the synthesis and characterization of new quinazolines as potential antimicrobial agents [38]

27. **Alagarsamy V *et al***., A series of novel 1-substituted-4-benzyl-4H-[1,2,4]triazolo[4,3-

a]quinazolin-5-ones were synthesized by the cyclization of 2-hydrazino-3-benzyl-3H-quinazolin-4-one with various one-carbon donors. When tested for their in vivo H1-antihistaminic activity on guinea pigs, all the test compounds protected the animals from histamine induced bronchospasm significantly [39].

28. **Mistry BD *et al.,*** have been synthesized 6–bromo–2–alkyl/aryl–3{[phenyl (phenyldiazenyl)methylene]amino}quinazolin–4(3*H*)–one reported their antimicrobial activity [40].

29. **Mosaad Sayed Mohamed *et al.,*** have been synthesized a series of new 5-(4-chlorophenyl)-9-iodo-3-substituted-l,2,4-triazolo[4,3-c]quinazoline and 2- (4-chlorophenyl)-6-iodo-4-substituted-quinazoline was prepared via several synthetic routes. The synthesized compounds were evaluated as anti-inflammatory agents through the carrageenin-induced paw edema test. The screening data revealed that nine of the tested compounds shown activity comparable to indomethacin [41].

30. **Terzio Glu *et al.,*** Two regioisomer series, 2-(3-ethyl-4(3H)-quinazolinone-2-ylmercaptoacetylhydrazono)-3-alkyl/3-aryl-5-methyl-4-thiazolidinones (12-21) and 2-arylimino-3-(3-ethyl-4(3H)-quinazolinone-2-ylmercaptoacetyla-mino)-5-methyl-4-thiazolidinones were synthesized and reported their anticonvulsant activity [42].

31. **Sona Jantova *et al.,*** The antibacterial activity of ten series of substituted quinazolines (157 derivatives) against bacterial strains *Escherichia coli* CCM 3988,*Pseudomonas aeruginosa* CCM 3955, *Bacillus subtilis* ATCC 6663 and *Staphylococcus aureus* CCM 3953 by micro dilution assay was investigated. The sensitivity of the Gram positive bacteria to the tested quinazolines was higher than that of Gram negative bacteria. The most effective of ten quinazoline structure series were condensed [1,2,4]triazoloquinazolines and 10H-[1,2,4]triazino[5,4-b]quinazolin-10-ones [43].

32. **Archana *et al.,*** have been synthesized derivatives of substituted quinazolinonyl-2-Oxo/thiobarbituric acid and their anticonvulsant activity was screened against maximal electroshock (MES) and pentylenetetrazole (PTZ) models [44].

33. **Abdel Ghany Aly El-helby *et al*.,** A series of halogenated derivatives, 3-methyl, 3-ethyl and 3-phenyl-6-mono and 6,8-disubstituted-3*H*-quinazolin-4-one derivatives was also synthesized and evaluated for anticonvulsant activity. Reduced anticonvulsant activity was recorded. Phenobarbitone sodium was used as a reference [45].

34. **Chioua R *et al*.,** The [4+2] cycloaddition between 2,4-diphenylpyrimidine ortho-quinodimethane and dimethyl acetylenedicarboxylate leads to 2,4-diphenylquinazoline-6,7-dicarboxylate .2,4-Diphenylfuro[3,4-g]quinazoline-6,8-dione is also obtained by basic hydrolysis of compound , followed by the closure of the resulting diacid in acetic anhydride [46].

35. **Archana *et al.,*** have been synthesized some thiadiazolyl and thiazolidinonyl quinazoline-4(3H)-ones screened them for anticonvulsant activity against maximal electroshock (MES) induced convulsions in animal models [47].

36. **V.K.Pandey *et al.,*** have been synthesized 1,4-disubstituted 3-[3'-(2'-phenyl-4'-oxo-quinazolinyl)]-2-azetidinones and reported their Antiferitility activity [48].

37. **Piyush Kumar *et al*.,** have been synthesized 6- substituted 6- substituted-2-phenyl-3-(5-substituted mercapto-1, 3, 4- thiadiazol-2-yl) quinazoline-4-(3H)-ones and reported their anti tubercular activity [49].

38. **H Mutlu Gençkal *et al*.,** reported new heterocyclic ligands, 2-(3-chloro-phenyl)-1, 2, 3, 4-tetrahydro-quinazoline-2-carbaldehyde oxime, (HL^1) and 2-(3-bromo-phenyl)-1,2,3,4-tetrahydro-quinazoline-2-carbaldehy- de oxime,(HL^2), and Co(III) complexes of their open-chain tautomer,($HL^{1\prime}$ and $HL^{2\prime}$), containing oxime, imine, and amine donor groups resulting from the reactions with Co(II) ion have been synthesized and characterized by spectral methods, elemental analysis, magnetic susceptibility, and thermal analysis (TG, DTG, and DTA) techniques [50].

39. **Jesus Sanmartín-Matalobos *et al.,*** Both experimental and computational studies were undertaken to elucidate the formation process of 3-tosyl-1,2,3,4-tetrahydroquinazoline from methanolic mother liquors of $Pd(L_{BS})\cdot 3H_2O$, where L_{BS} is the dianionic form of the imine ligand *N*-{2-[(8-hydroxyquinolin-2-yl) methyleneamino]benzyl}-4-

thylbenzenesulfonamide. Experimental studies have shown that the tetrahydroquinazoline is obtained by condensation of 2-tosyl amino methyl aniline and formaldehyde, which come from the acid-catalyzed hydrolysis of the imine ligand L_{BS} and metal-mediated aerobic oxidation of methanol, respectively. Computational studies have revealed relevant intermediates and key steps in the reaction pathway [51].

40. **CAI Suixiong *et al.,*** Disclosed are novel 1- (arylmethyl) -5, 6, 7, 8-tetrahydroquinazoline-2, 4-diones and analogs thereof, represented by the Formula I, wherein Ar, A, B, R_3-R_6 are defined herein. Compounds having Formula I are PARP inhibitors. Therefore, compounds of the invention may be used to treat clinical conditions that are responsive to the inhibition of PARP activity, such as cancer [52].

4. MATERIALS AND METHODS

4.1. Synthesis of tetrahydroquinazoline derivatives. In the present study we have attempted the synthesis of tetrahydroquinazoline derivatives using ethanol, para amino phenol and aldehyde.

Table 1: Chemicals and Reagents

S.No	Chemicals/ Reagents	Manufacturer

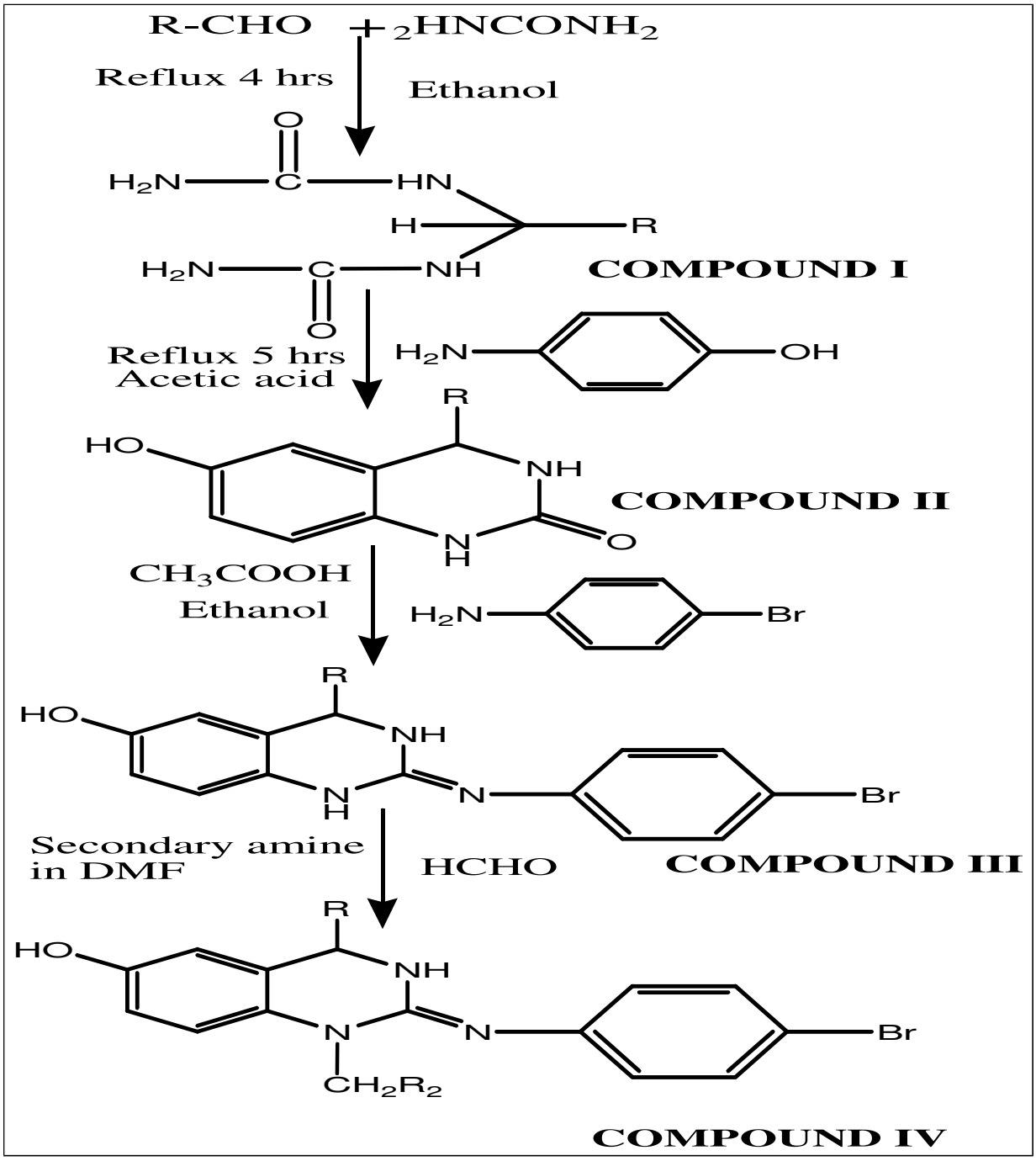
1	Vanillin	Sdfine chem. Ltd
2	Veratraldehyde	Sdfine chem. Ltd
3	P-Tolualdehyde	Sdfine chem. Ltd
4	3-Nitro Benzaldehyde	Sdfine chem. Ltd
5	Dimethyl amine	Sdfine chem. Ltd
6	Anisaldehyde	Spectrum
7	Urea	Merck
8	Ethanol	Changshu Yangyuan
9	Para amino phenol	Spectrum
10	Glacial acetic acid	Merck
11	Methanol	Qualigens
12	Diphenylamine	Spectrum
13	Morpholine	Sdfine chem. Ltd
14	Dimethyl Sulphoxide	Merck
15	Silica gel G precoated	Merck, Darmstadt
16	Aluminum sheets	Germany

4.2. Instruments

Table 2: Instrument List and Manufacturer

S.No	Instrument	Manufacturer
1	Digital electronic balance (BL- 220H)	KE Roy Electronics ,Varanasi.
2	Melting point apparatus	Shankar Scientific Chennai
3	FT-IR Spectroscopy	Shimadzu FT 8300, Japan.
4	NMR Spectroscopy	JEOL GSX 400, Japan.
5	Magnetic stirrer	Remi equipments, Chennai.
6	Mass Spectroscopy	JEOL GCmate, Japan.
7	UV Spectroscopy	+2060 Spectrophometer

4.3.SCHEME - I



R = Benzaldehyde

4.4. Synthesis of Compounds 1-4

Step – 1 Synthesis of 4-hydroxy-3-methoxy phenyl bis urea

A mixture of aldehydes (0.2 mol) and urea (0.4 mol) in absolute ethanol (100 ml) was heated under reflux for 4 hrs in such a manner that moisture air did not pass into the reaction mixture. Ethanol was removed by distillation and the residual solid was washed with water. The crude product was dried in and recrystallized using dilute methanol [53].

Step – 2 Synthesis of 4-substituted-2-keto-1,2,3,4-tetrahyeroquinazoline-6-ol

A mixture of 4-hydroxy-3-methoxyphenyl-bis-urea (0.2mol) and p-amino phenol (0.2 mol) were dissolved in glacial acetic acid (50 ml) by stirring and heating slowly. The acidic solution was subsequently heated under reflux for 5 hrs. The hot solution was cooled at room temperature and poured into ice-cold water (250 ml) and stirring vigorously for half an hour. It was filtered off and washed with water (3 × 25ml). The crude product was dried in *vacuo* and recrystallized using diluted ethanol.

Step–3Synthesis of,4-Substiuted-2- P-bromophenyl imimo-1,2,3,4-trahydroquinazoline-6-ol

A mixture of bromo aniline(0.2mol) and compound 2 in ethanol(30ml) containing 3-4 drops of glacial acetic acid was heated for half an hour and left overnight at room temperature. The solid product thus obtained was recrystallized with methanol.

Step–4 Synthesis of [(4-bromo phenylimino)-1,4-disubtituted-1,2,3,4-tetrahydro quinazoline-6-ol)].

A mixture of compounds 3 (0.01mol) was suspended in a minimum quantity (10ml) of dimethyl formamide (DMF). To this solution slight more than 0.01mol of formaldehyde and various secondary amines were added with vigorous stirring. The reaction mixtures were

heated on water bath for 20 minutes and left overnight. The product thus obtained was re-crystallized from ethanol solvent [54]

4.5. Target Protein for Docking Study [55]

4.5.1. Polo-Like Kinase 1 (PLK1)

Antimitotics form the basis of the therapy for patients with both solid tumors and hematological malignancies. However, current antimitotic drugs affect both dividing and non-dividing cells. One of the emerging next generation antimitotic targets is Polo-like kinase 1 (PLK1). Among the four members of PLK family, PLK1 is the best characterized and it is recognized to be a key component of the cell cycle control machinery with important roles in the mitotic entry, centrosome duplication, bipolar mitotic spindle formation, transition from metaphase to anaphase, cytokinesis and maintenance of genomic stability. PLK1 is often over-expressed in many different tumor types and over-expression often correlates with poor prognosis. As an antimitotic target, PLK1 is only expressed in dividing cells while it is not expressed in differentiated postmitotic cells like neurons, where instead expression of PLK2 and PLK3 was reported. This indicates a potentially better safety profile for a PLK1 specific inhibitor. Thus, PLK1 is thought to be a promising target for anti-cancer therapy and indeed some PLK1 inhibitors are currently under evaluation in clinical trials.

Active site chosen: Cys 67

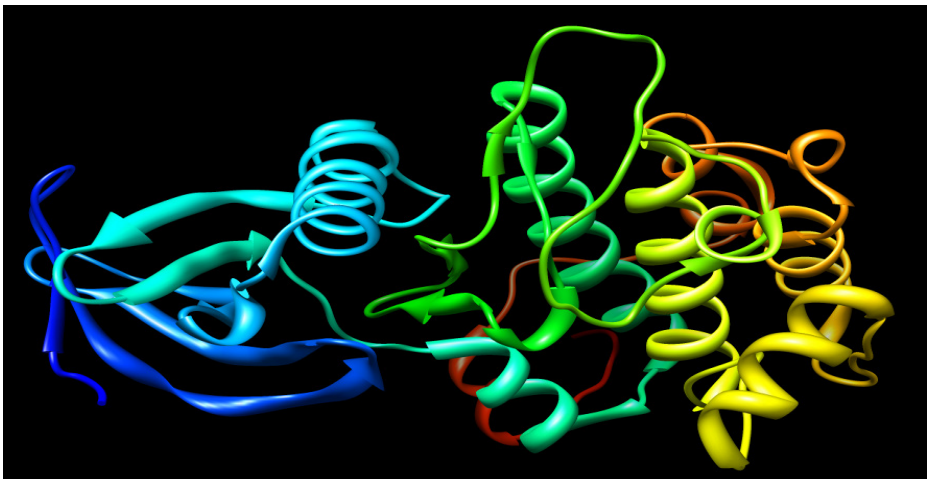


Figure 2: Structure of PLK1

Figure 3: Docking of compound $4a_1$ with PLK-1

Figure 4: Docking of compound $4a_2$ with PLK-1

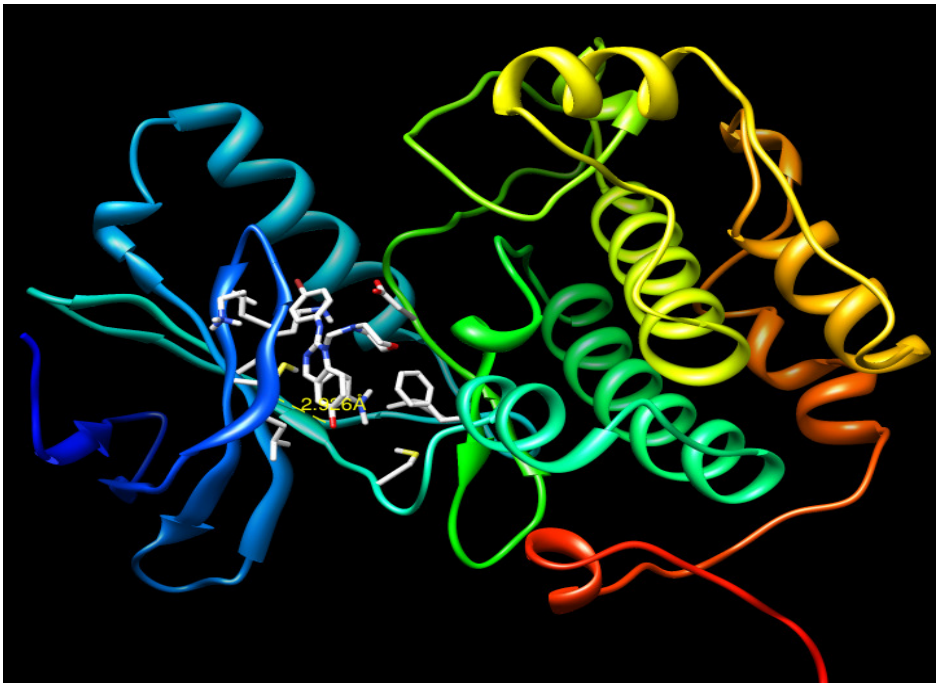


Figure 5: Docking of compound $4a_3$ with PLK-1

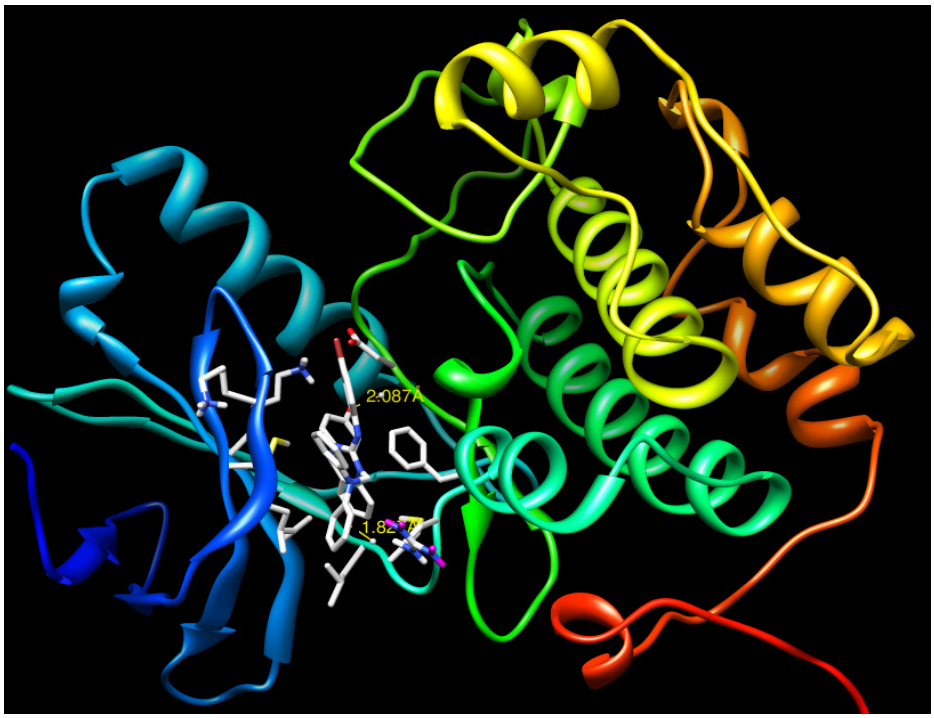


Figure 6: Docking of compound $4a_4$ with PLK-1

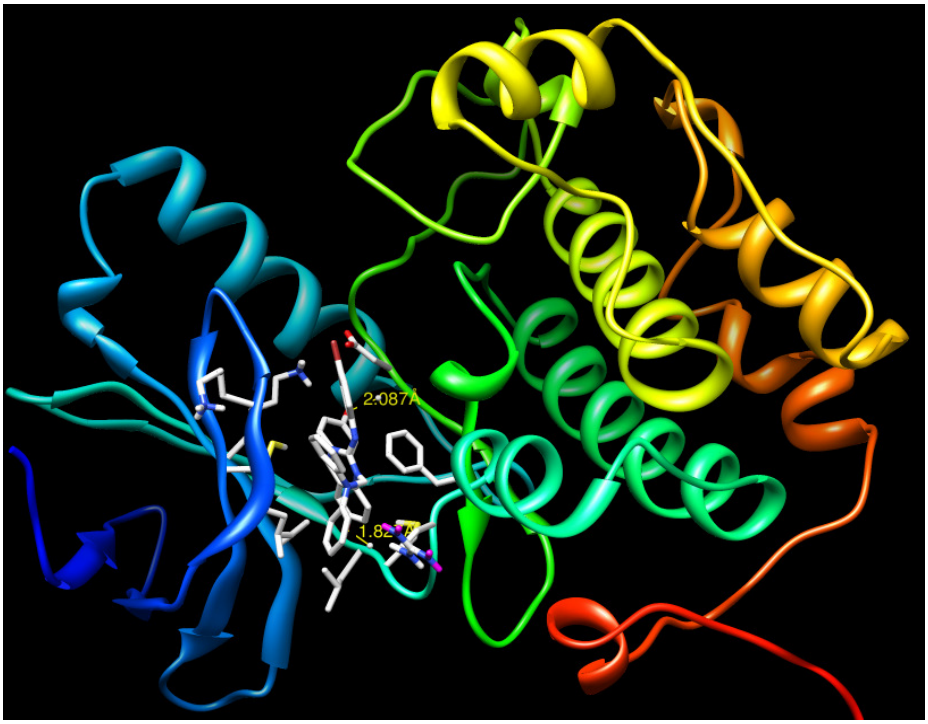


Figure 7: Docking of compound $4a_5$ with PLK-1

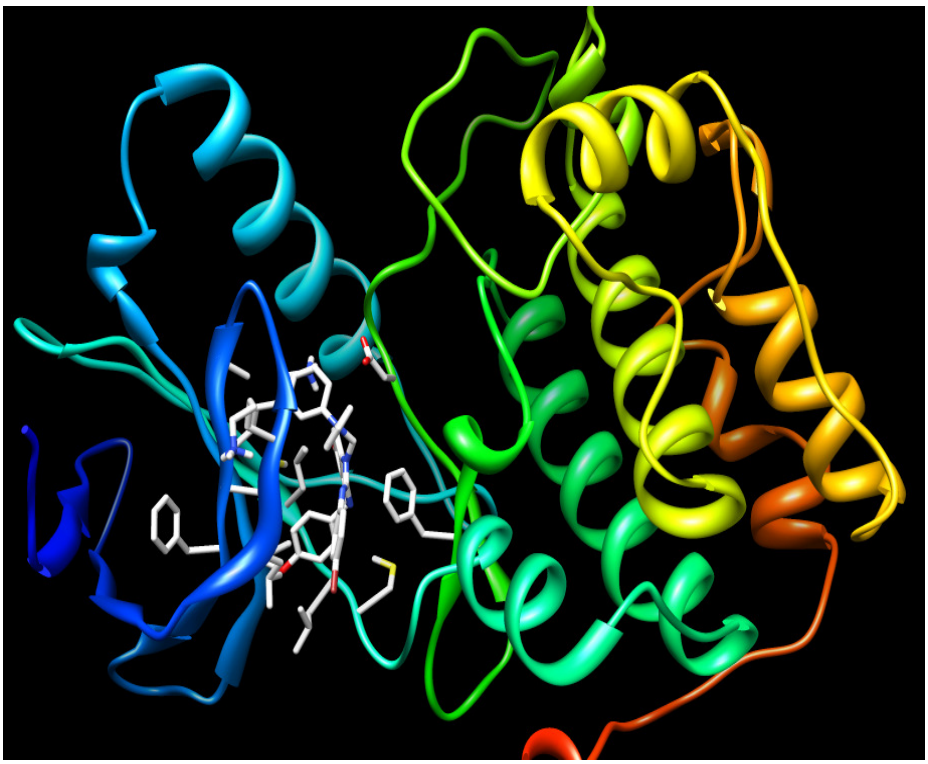


Figure 8: Docking of compound $4a_6$ with PLK-1

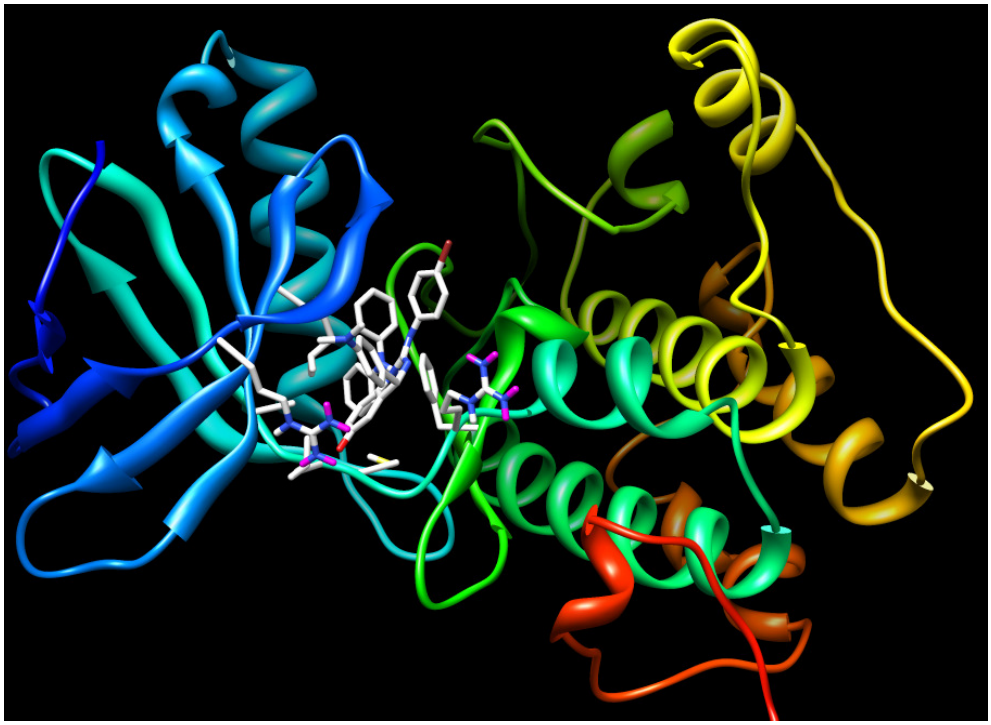


Figure 9: Docking of compound $4a_7$ with PLK-1

Figure 10: Docking of compound $4a_8$ with PLK-1

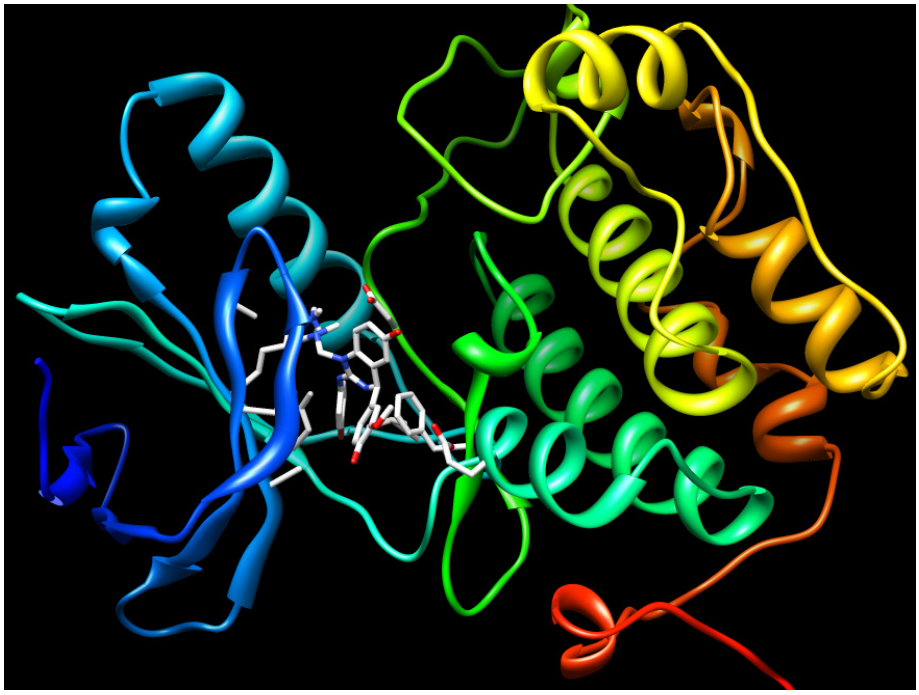


Figure 11: Docking of compound $4a_9$ with PLK-1

Figure 12: Docking of compound $4a_{10}$ with PLK-1

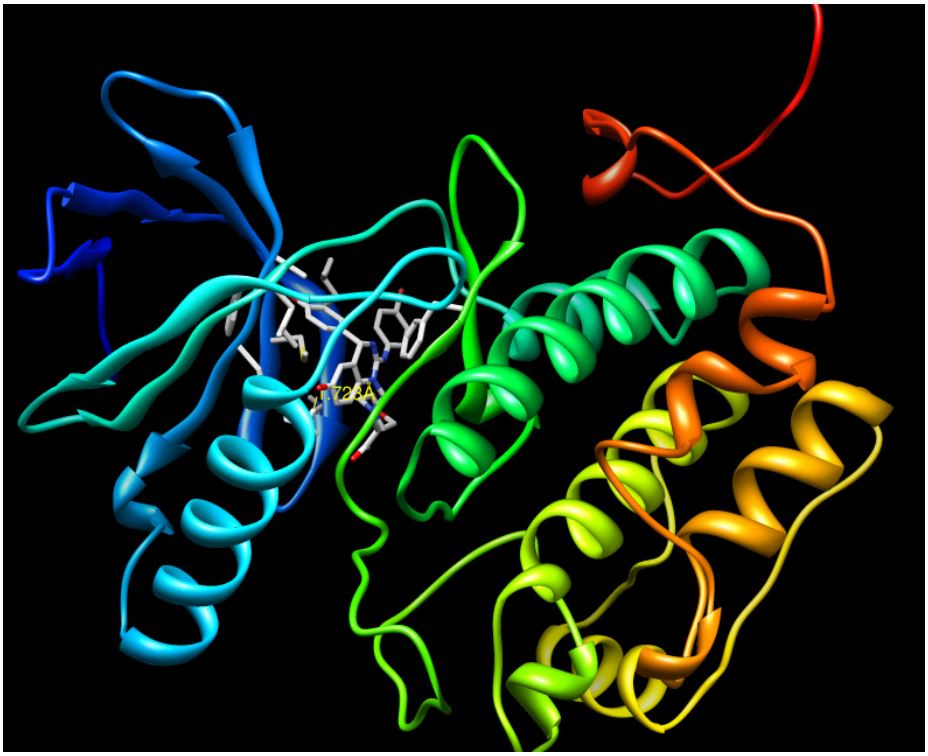


Figure 13: Docking of compound $4a_{11}$ with PLK-1

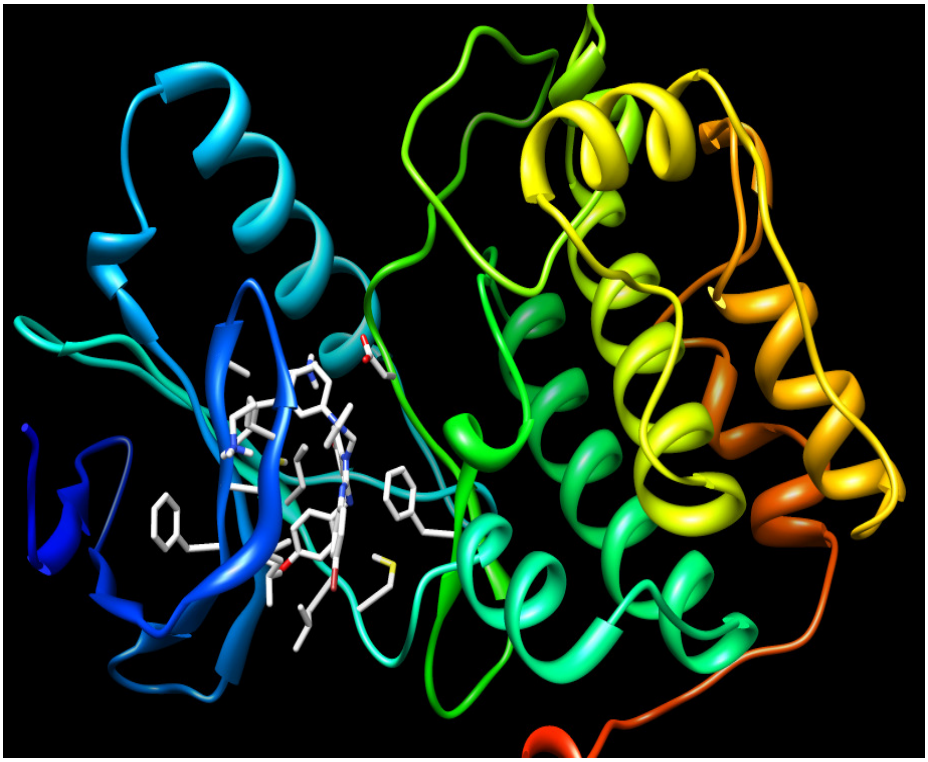


Figure 14: Docking of compound $4a_{12}$ with PLK-1

5.BIOLOGICAL SCREENING

5.1. Hepato Protective activity of synthesized tetra hydroquinazoline derivatives.

5.1.1. Animal Grouping and Maintenance

Male Wistar albino rats weighed 200 ± 20 g were utilized for this study. They were housed in polypropylene cages under standard laboratory conditions (12-h light/ 12-h dark cycle, 21 ± 2 ºC, and relative humidity 55 %). The animals were given standard rodent pellets and water *ad labium*. The rats were acclimatized to laboratory condition for 7 days before the commencement of experiment. The hepatoprotective study was carried out by the Chromosoft Infotech, Chennai as per the guidelines set by Organization for Economic Co-operation and Development (OECD) received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA NO. 8926/242/PAHR).

On eighth day, the rats were sacrificed by overdose of anaesthetics, liver was separated and used for estimation of biochemical parameters.

5.1.2. Experimental procedure

In vitro study was done by using liver slice culture system following the protocol developed by Wormser and Ben Zakine (1990) with slight modification. Rat liver was perfused heavily by using Modified Hank's Medium to remove all the blood clots. The liver was then cut into small square slices, weighing 5–6 mg, and was cultured in 24 well plates (20–22 slices weighing about 100–120mg/well) using MEM 199 medium supplemented with 0.1-g/l penicillin, 0.07-g/l streptomycin and 0.2% BSA. Liver slices were incubated in this condition

for 4 h at 37^0 C in 95% O_2 / 5% CO_2. The medium was changed at 2 h, this time gap was given to recover the shock from surgery. The control slices were kept in culture medium only, ethanol (50 mg/ml) or ethanol plus $4a_1$ and $4a_4$ (2.5, 5.0 and 10.0 mg/ml) or ethanol plus Silymarin (1.0, 2.0 and 3.0 mg/ml) were added to the incubation medium where mentioned.

On termination of incubation at 4 h, the medium was collected and subjected to the determination of SGOT and SGPT for the assessment of liver damage.

The liver slices from different incubations were separately collected, washed repeatedly and then homogenized in ice-cold phosphate (50mM, pH 7.4) buffer; the homogenate for each incubation was subjected to the estimation of lipid peroxidation.

Since co-treatment of $4a_1$ and $4a_4$ and alcohol for 12 days showed an optimum response, the rest of the experiments were conducted for 12 days. To examine the efficacy of $4a_1$ and $4a_4$ with a standard drug for liver damage treatment, I have selected Silymarin, at a dose of 5mg/kg body weight for a single dose each day, keeping control and $4a_1$ and $4a_4$ treated group in a similar manner as described above. PBS using Potter Elvehjem homogenizer and then subjected to differential centrifugation to separate cytosolic and microsomal fractions according to the method. Protein estimation of the samples was done by using the method of Lowry.

5.1.3. Serum Glutamate Oxaloacetate Transaminase

Preparation of Diluted Serum: Fresh blood was allowed to clot and serum was collected. Serum was then diluted with physiological saline in 1: 10 ratio. 0.2 – ml diluted serum was taken in a test tube marked sample and to it 1 ml substrate buffer solution was added. It was then mixed by inversion and incubated at 37^0 C for exactly 60 minutes. In a tube marked blank 0.2 ml diluted serum was taken and to it 1 ml substrate buffer solution was added. It was mixed well but no incubation was done [56-59].

In tubes marked standard different volumes of working standard solution were taken, *viz*. 0.1 ml, 0.2 ml, 0.3 ml. Then GDW was added in a way making the volume of each tube 2.2 ml. To “sample”, “blank” and “standard” tubes 1 ml ketone reagent was added and kept at room temperature for 15 minutes. To tubes “sample” and “blank” 5 ml in each and to “standard” 10 ml of 0.4 (N) NaOH was added, mixed thoroughly and kept at room temperature for 20

minutes [60-62]. Optical densities of all the solutions were measured at 530 nm against the blank solution. A standard curve was plotted with standard values and the unit of enzyme present per 100 ml serum sample was calculated from curve [63].

5.1.4. Serum Glutamate Pyruvate Transaminase (SGPT)

In the tube marked sample 1 ml substrate buffer solution was taken and to it 0.2 ml diluted serum (prepared as in SGOT estimation) was added, mixed thoroughly by inversion and incubated at 37^0 C for 60 minutes. In the tube marked blank 1 ml substrate buffer solution and 0.2 ml diluted serum were added but no incubation was done [63-68]. 1 ml ketone reagent was added to both the tubes, which were then kept at room temperature for 15 minutes. 5 ml 0.4 (N) NaOH was added into each tube, mixed well and kept at room temperature for 20 minutes. Optical density of the experimental solution was read against the blank at 530 nm. From the standard curve (plotted similarly in SGOT estimation), the unit of enzyme present per 100 ml of serum was calculated [68-75].

5.1.5. Estimation of TBARS

The level of lipid peroxidation as measured by TBARS was determined according to the method of Buege and Aust (1978). Briefly, 1ml of the microsomal fraction (containing 1mg/ml protein) was mixed with 2ml of the TBA–TCA–HCl reagent and vortexed thoroughly. After heating for 15min in a boiling water bath, the samples were allowed to cool and then centrifuged at 1000×g for 10min to remove the flocculants. The supernatant was checked for TBARS content as a measure of lipid peroxidation.

5.1.6. Estimation of protein carbonyl content

Protein carbonyl content was determined according to Levine et al. (1990), using 0.8 ml of the cell free homogenate (10% homogenate centrifuged at 500×g for 10min) in 50mM sodium phosphate buffer, pH 7.4. The protein was suspended with 0.5 ml of 2, 4, dinitro phenylhydrazine and after washing in ethanol–ethyl acetate mixture (1:1), it was dissolved

with 0.6 ml of guanidine–Protein carbonyl content was determined from the extinction coefficient at 362nm (ε = 22,000M−1 cm−1).

5.1.7. Catalase

Catalase activity was assayed by the method of Aebi (1984), as modified by Kawamura (1999). 20l of the cytosolic fraction was added to 980l assay buffer containing 50mM Tris–HCl (pH 8.0), 9mM H_2O_2 and 0.25mM EDTA to constitute the assay volume of 1ml. The decrease in absorbance of that assay mixture was recorded at 240 nm for 1min. The results were expressed as unit catalase/mg protein.

5.1.8. Measurement of PNP–UGT activity

p-Nitrophenol (PNP) UGT activity was determined by the method described by Bock as modified by Viollon- Abadie. Briefly, the reaction mixture consisted of 5mM UDP-glucuronic acid, 1mM PNP and microsomal sample without (native) or with (activated) 0.05% Triton X-100 in a final volume of 200 litre. The reaction mixture was incubated at 37 °C for 30 min and then stopped by the addition of 3.8 ml of NaOH (0.1M). The PNP glucuronidation was quantified by measuring the decrease in absorbance at 405nm (ε=18mM−1 cm−1).

6. RESULTS AND DISCUSSION

The synthesized compound 4 [(4-Bromo phenylimino)-1,4-disubstituted-1,2,3,4-tetrahydroquinazoline-6-ol)] was used for the synthesis of various tetrahydroquinazoline derivatives was shown in figure 15. Further docking studies was done with different substrates in R and R^1 positions was shown in table 3.

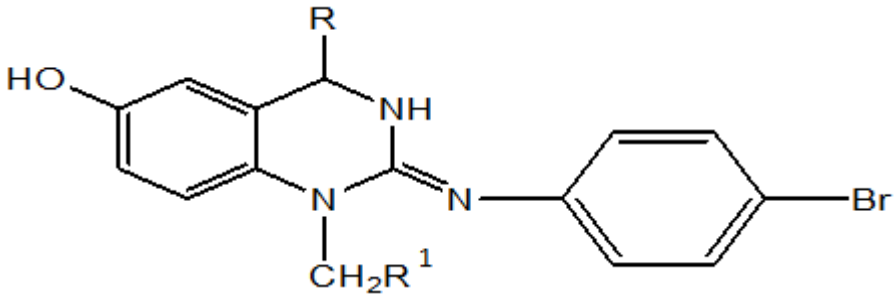
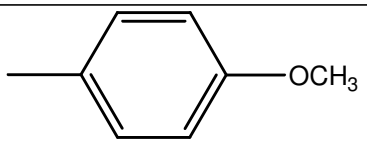
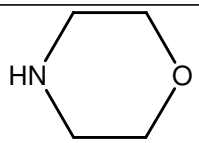
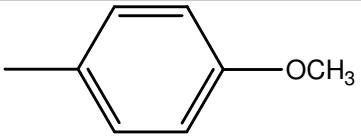
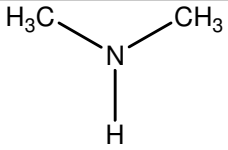
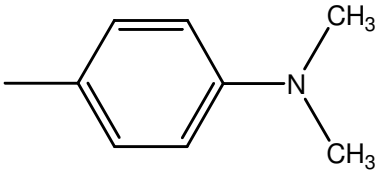
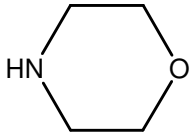
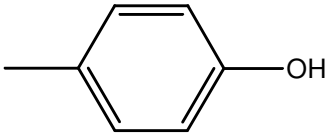
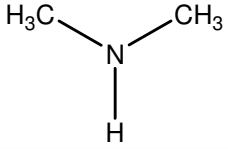
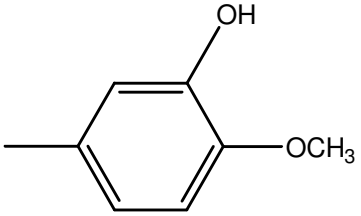
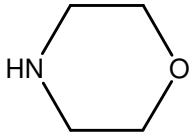
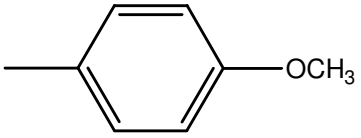
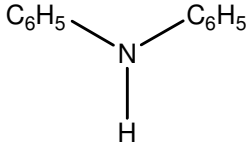


Figure 15: Synthesized compound 4

Table 3: Various Substitutions of the Synthesized Compounds 4a1- 4a12

S.No	Compound code	R	R^1
1.	$4a_1$		
2.	$4a_2$		
3.	$4a_3$		
4.	$4a_4$		
5.	$4a_5$		
6.	$4a_6$		

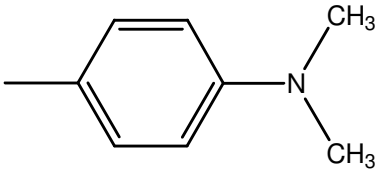
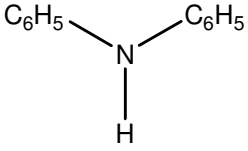
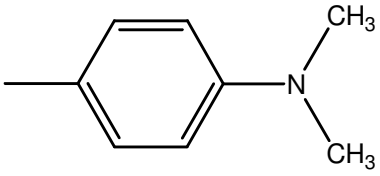
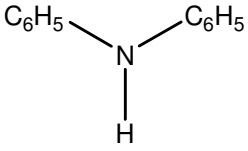
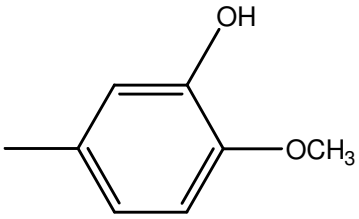
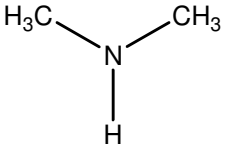
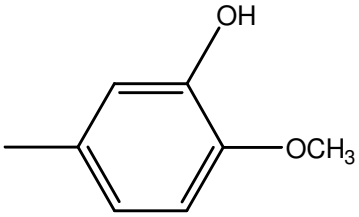
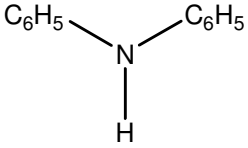
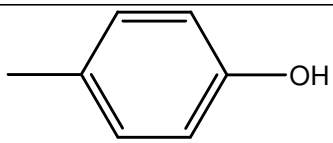
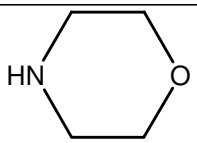
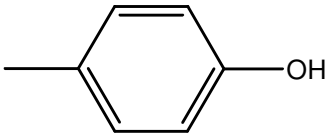
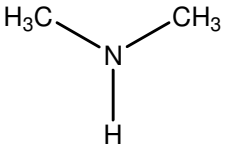
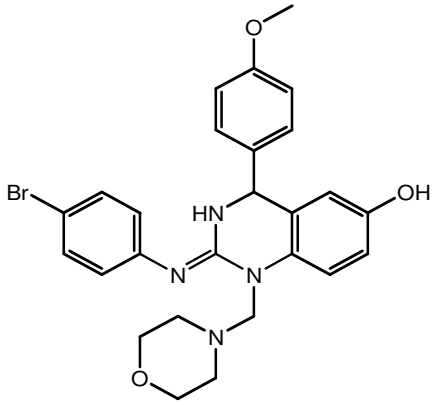
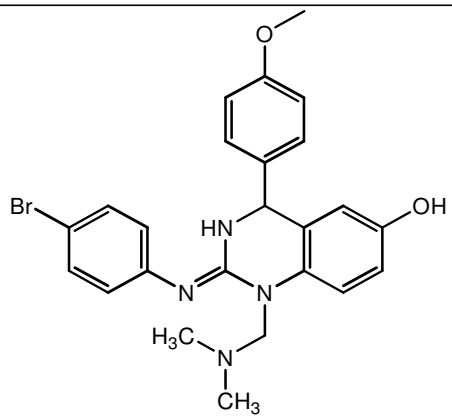
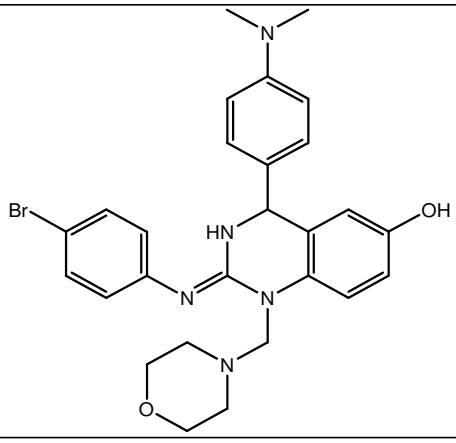
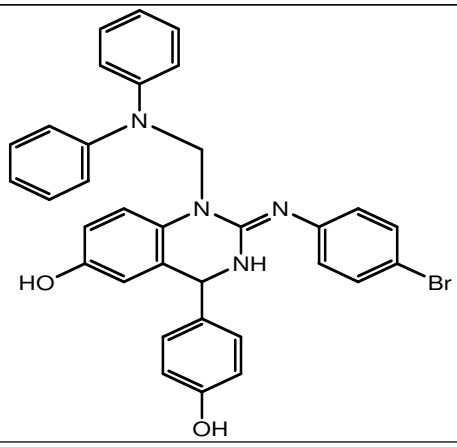
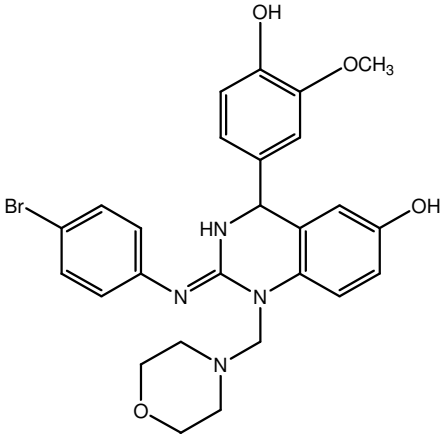
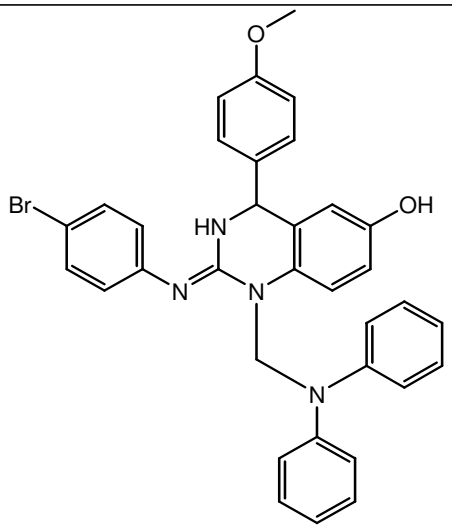
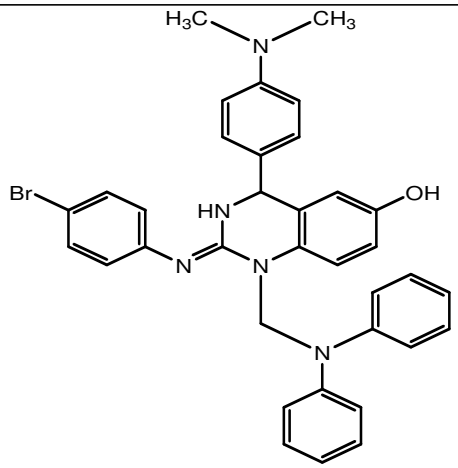
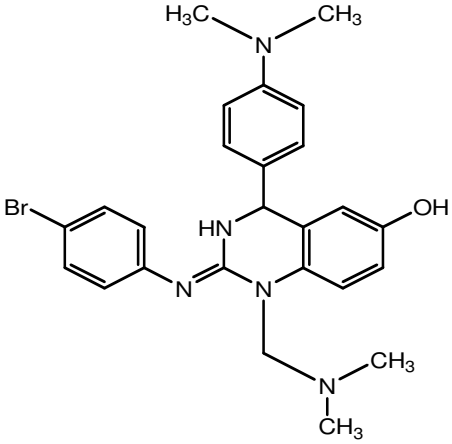
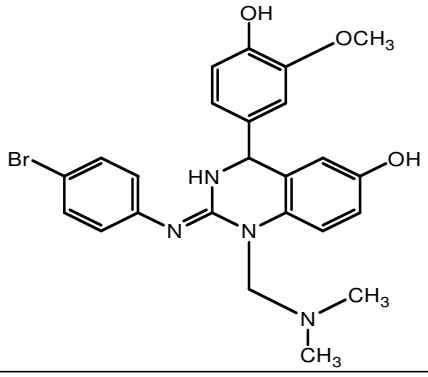
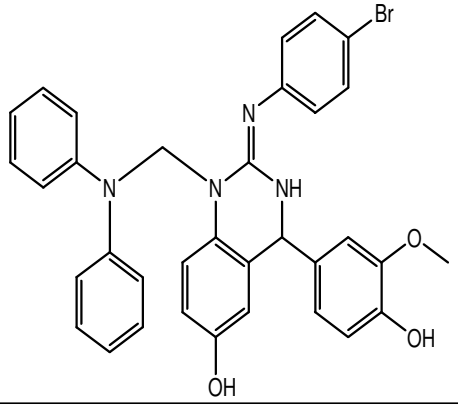
7.	$4a_7$		
8.	$4a_8$		
9.	$4a_9$		
10.	$4a_{10}$		
11.	$4a_{11}$		
12.	$4a_{12}$		

Table 4: List of Synthesized Compounds

S.No	Compound code	Name	Structure
1.	$4a_1$	(E)-2-(4-bromophenylimino)-4-(4-methoxyphenyl)-1-(morpholinomethyl)-1,2,3,4-tetrahydroquinazolin-6-ol	
2.	$4a_2$	(E)-2-(4-bromophenylimino)-1-((dimethylamino)methyl)-4-(4-methoxyphenyl)-1,2,3,4-tetrahydroquinazolin-6-ol	
3.	$4a_3$	(E)-2-(4-bromophenylimino)-4-(4-(dimethylamino)phenyl)-1-(morpholinomethyl)-1,2,3,4-tetrahydroquinazolin-6-ol	

4.	$4a_4$	(E)-2-(4-bromophenylimino)-1-((diphenylamino)methyl)-4-(4-hydroxyphenyl)-1,2,3,4-tetrahydroquinazolin-6-ol	
5.	$4a_5$	(E)-2-(4-bromophenylimino)-4-(4-hydroxy-3-methoxyphenyl)-1-(morpholinomethyl)-1,2,3,4-tetrahydroquinazolin-6-ol	
6.	$4a_6$	(E)-2-(4-bromophenylimino)-1-((diphenylamino)methyl)-4-(4-methoxyphenyl)-1,2,3,4-tetrahydroquinazolin-6-ol	

7.	$4a_7$	(E)-2-(4-bromophenylimino)-4-(4-(dimethylamino)phenyl)-1-((diphenylamino)methyl)-1,2,3,4-tetrahydroquinazolin-6-ol	
8.	$4a_8$	(E)-2-(4-bromophenylimino)-1-((dimethylamino)methyl)-4-(4-(dimethylamino)phenyl)-1,2,3,4-tetrahydroquinazolin-6-ol	
9.	$4a_9$	(E)-2-(4-bromophenylimino)-1-((dimethylamino)methyl)-4-(4-hydroxy-3-methoxyphenyl)-1,2,3,4-tetrahydroquinazolin-6-ol	
10.	$4a_{10}$	(E)-2-(4-bromophenylimino)-1-((diphenylamino)methyl)-4-(4-hydroxy-3-methoxyphenyl)-1,2,3,4-tetrahydroquinazolin-6-ol	

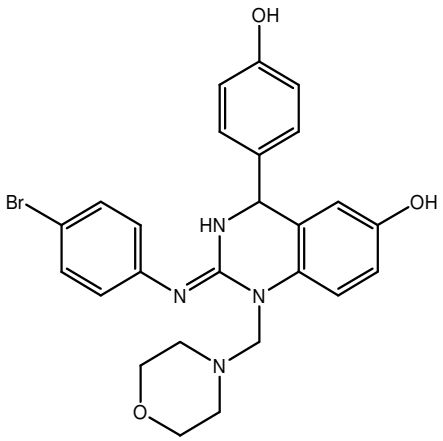
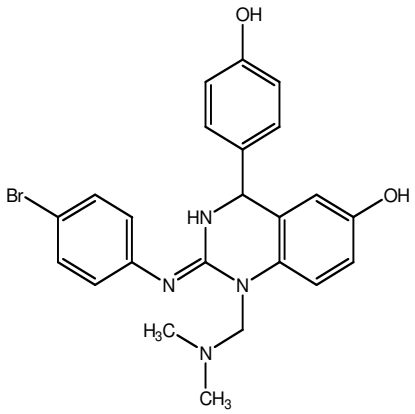
11.	$4a_{11}$	(E)-2-(4-bromophenylimino)-4-(4-hydroxyphenyl)-1-(morpholinomethyl)-1,2,3,4-tetrahydroquinazolin-6-ol	
12.	$4a_{12}$	(E)-2-(4-bromophenylimino)-1-((dimethylamino)methyl)-4-(4-hydroxyphenyl)-1,2,3,4-tetrahydroquinazolin-6-ol	

Table 6: Application of lipinski rule of 5 to our ligand test set

S.No	Ligand	Molecular weight	Number of Hydrogen bond acceptors	Number of Hydrogen bond donors	Logp (oct/wat)	Lipinski Violations
1	$4a_1$	523.431	7	2	5.024	2
2	$4a_2$	481.394	6	2	5.178	1
3	$4a_3$	536.474	7	2	5.069	2
4	$4a_4$	591.509	6	3	8.036	2
5	$4a_5$	539.43	8	3	4.309	1
6	$4a_6$	618.579	6	2	8.481	2
7	$4a_7$	605.536	6	2	8.453	2
8	$4a_8$	494.437	6	2	5.223	1
9	$4a_9$	497.393	7	3	4.46	0
10	$4a_{10}$	546.798	0	0	9.497	2
11	$4a_{11}$	509.404	7	3	4.488	1
12	$4a_{12}$	446.367	6	3	4.642	0

Chromatography Studies of Synthesized Compound

6.1. Thin Layer Chromatography

Thin Layer Chromatography or TLC is a solid-liquid form of chromatography here the stationary phase is a polar absorbent and the mobile phase can be a single solvent or Combination of solvents. TLC is inexpensive technique and quick that can be used for determine the number of components in a mixture, verify a substance's identity, monitor the process of a reaction, determine appropriate condition for column chromatography, analyze the fractions obtained from column chromatography.

6.1.1. Materials and Methods

1. Preparation of plates

Silica gel G was mixed in a glass mortar to smooth consistency with the requisite amount of water and slurry was quickly transferred to the spreader. The mixtures have been spread over the plates in thickness of 0.2 mm and allow setting into a suitable holder and after 30 minutes, plates were dried at 120ºC, for further activation of the absorbent.

2. Sample application

About 2 mm of absorbent from the edge of plate was removed to give sharply defined edges. 2-5 µl volumes of synthesized compounds were spotted with the help of capillary tubes, just above 2 cm of the bottom of coated plates.

3. Development chamber

The chromatographic chamber was lined with filter paper dipping into mobile phase so as to maintain the atmospheric saturation with solvent vapors in the chamber. The solvent front was allowed to rise to distance of about 12cm from the base line on the plate was removed from the tank and allowed to dry in the air.

4. Solvent system

The choice of best developing solvent is one of the most important decisions in practical TLC by review of literature survey on by knowing nature of compounds, this solvent system used is toluene : ethyl acetate : formic acid (5:4:1).

5. Detection of components

The spots were visualized under Iodine chamber

6.1.2. Lipinski's Rule

Lipinski's rule says that, in general, an orally active drug has no more than one violation of the following criteria:

- Not more than 5 hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms)
- Not more than 10 hydrogen bond acceptors (nitrogen or oxygen atoms)
- A molecular weight under 500 Daltons
- An octane-water partition coefficient log *P* of less than 5.

Note that all numbers are multiples of five, which is the origin of the rule's name.

6.1.3. Improvements

To evaluate drug likeness better, the rules have spawned many extensions, for example one from a 1999 paper by Ghose et al,

- Partition coefficient log *P* in -0.4 to +5.6 range
- Molar refractivity from 40 to 130
- Molecular weight from 160 to 480
- Number of atoms from 20 to 70

At Mol inspiration we believe that the strategy which leads to success is not a universal drug-likeness score, but focus on particular drug classes and development of specific activity score for each of these classes. The method we implemented uses sophisticated Bayesian statistics to compare structures of representative ligands active on the particular target with structures of inactive molecules and to identify substructure features (which in turn determine physicochemical properties) typical for active molecules.

With the Mol inspiration virtual screening toolkit miscreant one can easily develop a screening engine for arbitrary target, provided that several active legends (in the extreme case only single ligand) are known. With the trained model it is possible to screen large libraries of hundreds of thousands of molecules in less than hour, to identify molecules with highest chance to become active drugs or pesticides.

Expert system for calculation of drug likeness score towards GPCR ligands, ion channel modulators, kinase inhibitors, nuclear receptor ligands, protease inhibitors and other enzyme targets based on Mol inspiration technology may be tested on-line.

Table 6: Application of lipinski rule of 5 to our ligand test set

S.No	Ligand	Molecular weight	Number of Hydrogen bond acceptors	Number of Hydrogen bond donors	Logp (oct/wat)	Lipinski Violations
1	$4a_1$	523.431	7	2	5.024	2
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5	$4a_5$	539.43	8	3	4.309	1
6	$4a_6$	618.579	6	2	8.481	2
7	$4a_7$	605.536	6	2	8.453	2
8	$4a_8$	494.437	6	2	5.223	1
9	$4a_9$	497.393	7	3	4.46	0
10	$4a_{10}$	546.798	0	0	9.497	2
11	$4a_{11}$	509.404	7	3	4.488	1
12	$4a_{12}$	446.367	6	3	4.642	0

Table 7: Molinspiration bio activity score of $4a_1$ – $4a_{12}$

S.No	Ligand	GPCR ligand	Ion channel modulator	Kinase Inhibitor	Nuclear Receptor ligand	Protease inhibitor	Enzyme inhibitor
1	$4a_1$	-0.11	-0.17	-0.35	-0.51	-0.34	-0.16
2	$4a_2$	-0.10	-0.10	-0.36	-0.47	-0.36	-0.13
3	$4a_3$	-0.08	-0.14	-0.30	-0.46	-0.31	-0.13
4	$4a_4$	-0.03	-0.22	-0.25	-0.32	-0.22	-0.11
5	$4a_5$	-0.10	-0.17	-0.32	-0.52	-0.36	-0.15
6	$4a_6$	-0.07	-0.39	-0.33	-0.40	-0.22	-0.20
7	$4a_7$	-0.07	-0.33	-0.32	-0.38	-0.24	-0.18
8	$4a_8$	-0.07	-0.07	-0.32	-0.42	-0.33	-0.10
9	$4a_9$	-0.09	-0.10	-0.33	-0.49	-0.38	-0.12
10	$4a_{10}$	-0.07	-0.44	-0.39	-0.11	-0.10	-0.23
11	$4a_{11}$	-0.08	-0.12	-0.34	-0.51	-0.31	-0.13
12	$4a_{12}$	-0.07	-0.05	-0.34	-0.46	-0.33	-0.10

6.1.4. SPECTRAL DATA

Table 8: IR Spectral Data of Compound $4a_1$

S.NO	FREQUENCY	MODE OF VIBRATION
1	1448	m CH_3 bending
2	1405	m O-H in plane bending and C-O Stretching
3	3215	m N-H Stretching
4	1300-1253	s Asymmetric C-O-C stretching
5	1339-1314	m C-N stretching
6	589-514	C-Br stretching
7	1680	C=N stretching
8	1589	C=C stretching
9	976	s C-H out of plane bending
10	3070	C-H stretching

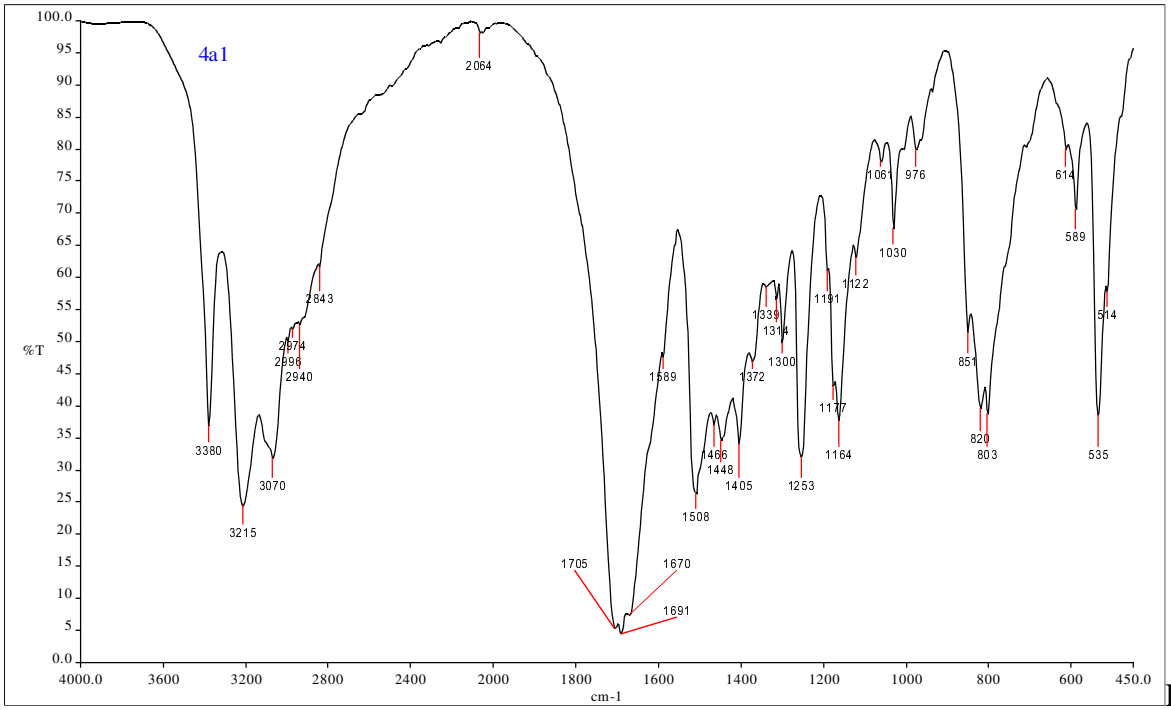

Figure 16: IR Spectrum of the Compound $4a_1$

Table 9: IR Spectral Data of Compound $4a_2$

S.NO	FREQUENCY	MODE OF VIBRATION

1	1448	m CH3 bending
2	1405	m (O-H in plane bending and C-O Stretching
3	3215	m (N-H Stretching)
4	1122	S (Asymmetric C-O-C stretching)
5	1314	m (C-N stretching)
6	589-514	C-Br stretching
7	1680	C=N stretching
8	1589	C=C stretching
9	820 – 803	s C-H out of plane bending
10	3070	C-H stretching

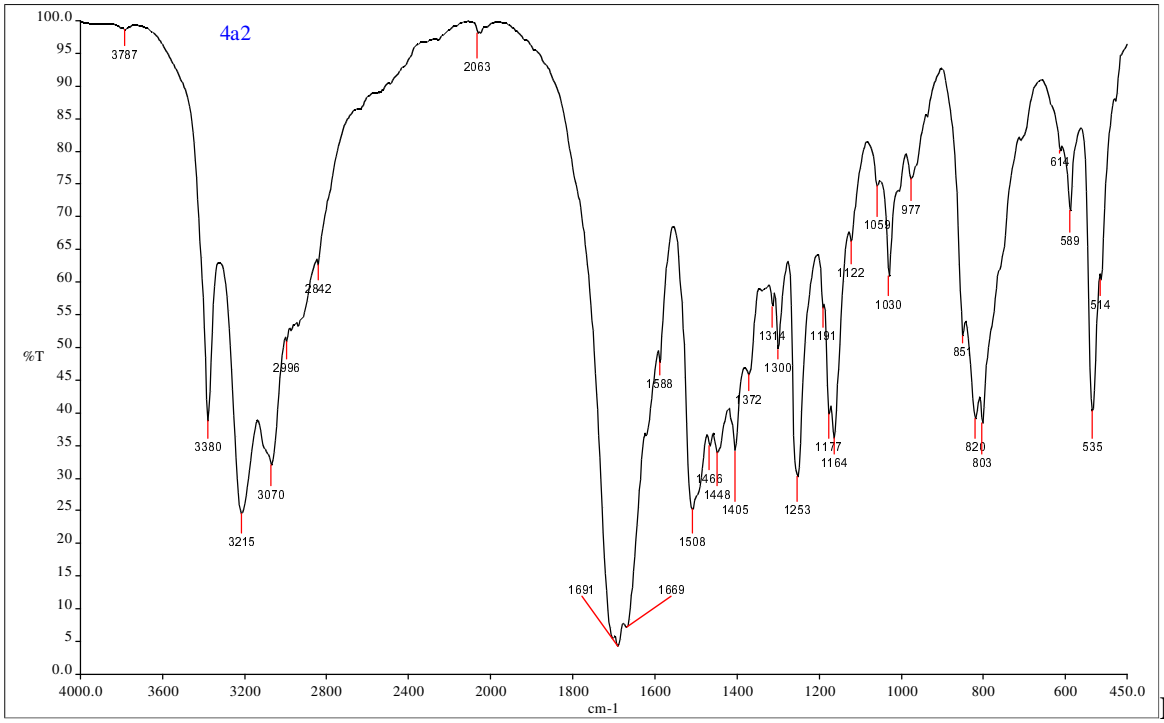

Figure 17: IR Spectrum of the Compound 4a$_2$

Table 10: IR Spectral Data of Compound 4a$_3$

S.NO	FREQUENCY	MODE OF VIBRATION
1	2810	m CH3 bending

2	1410 – 1400	m (O-H in plane bending and C-O Stretching
3	3430	m - w(N-H Stretching)
4	1120	s (Asymmetric C-O-C stretching)
5	1314	m (C-N stretching)
6	593-509	C-Br stretching
7	1659	C=N stretching
8	1599	C=C stretching
9	834 – 815	s C-H out of plane bending
10	1227 – 1003	w C-H in plane bending

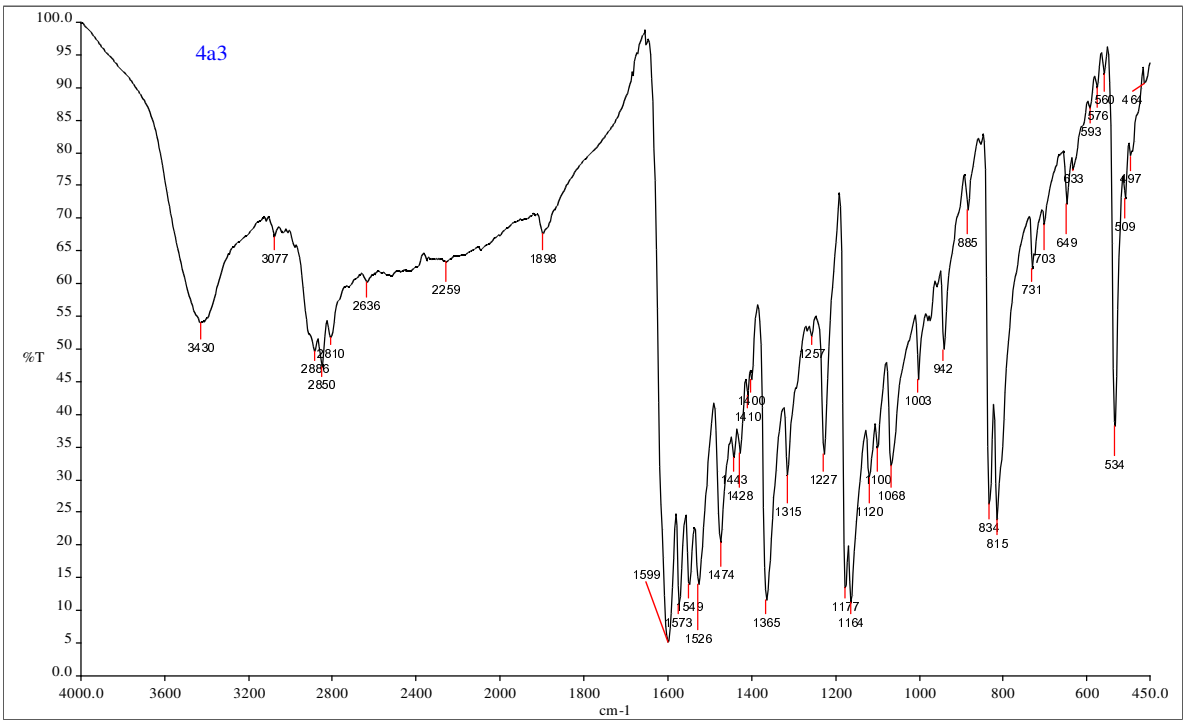

Figure 18: IR Spectrum of the Compound 4a$_3$

Table 11: IR Spectral Data of Compound 4a$_4$

S.NO	FREQUENCY	MODE OF VIBRATION
1	2810	m CH3 bending
2	1416	m (O-H in plane bending and C-O Stretching
3	3392	m - w(N-H Stretching)

4	1120	s (Asymmetric C-O-C stretching)
5	1314	m (C-N stretching)
6	589 – 502	C-Br stretching
7	1659	C=N stretching
8	1594	C=C stretching
9	816	s C-H out of plane bending
10	1224 – 1005	w C-H in plane bending

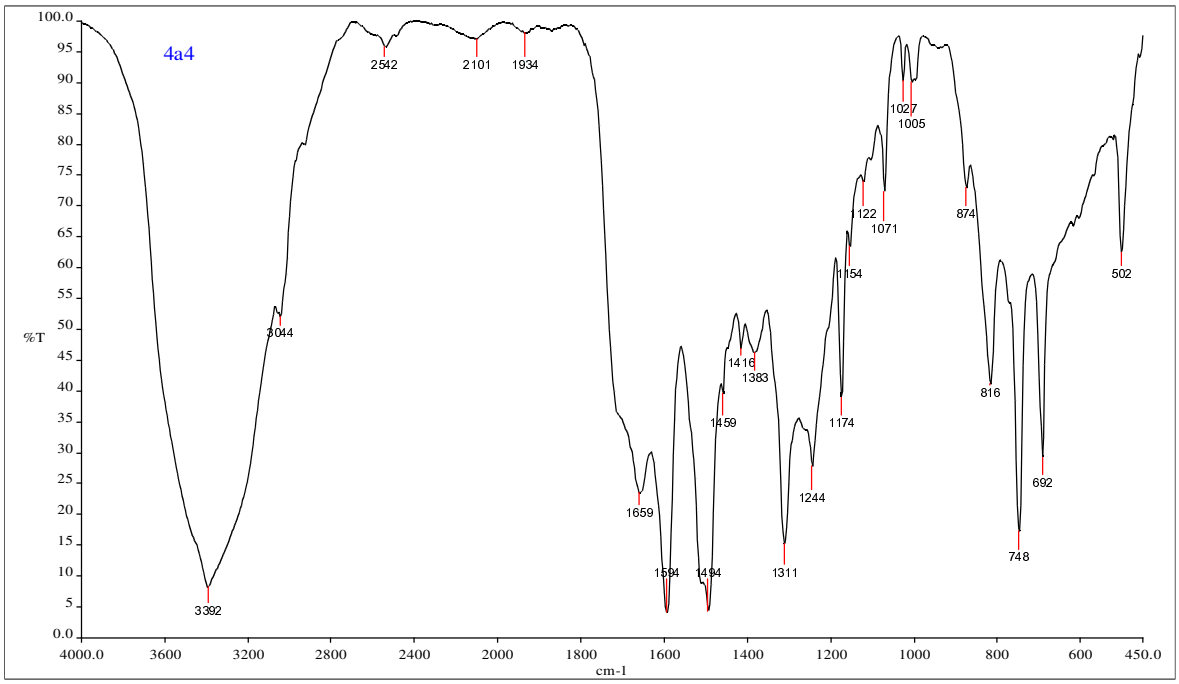

Figure 19: IR Spectrum of the Compound $4a_4$

Table 12: Mass Spectral Data of Compound $4a_1$ and $4a_4$

S.No.	Compound Code	Mass fragmentation pattern
1	$4a_1$	523.13 (28.5%), 524.12 (97.12%), 522.13 (100%)
2	$4a_4$ 593.13 (36.2%), 592.13	

	(97.8%), 590.13 (100%)	

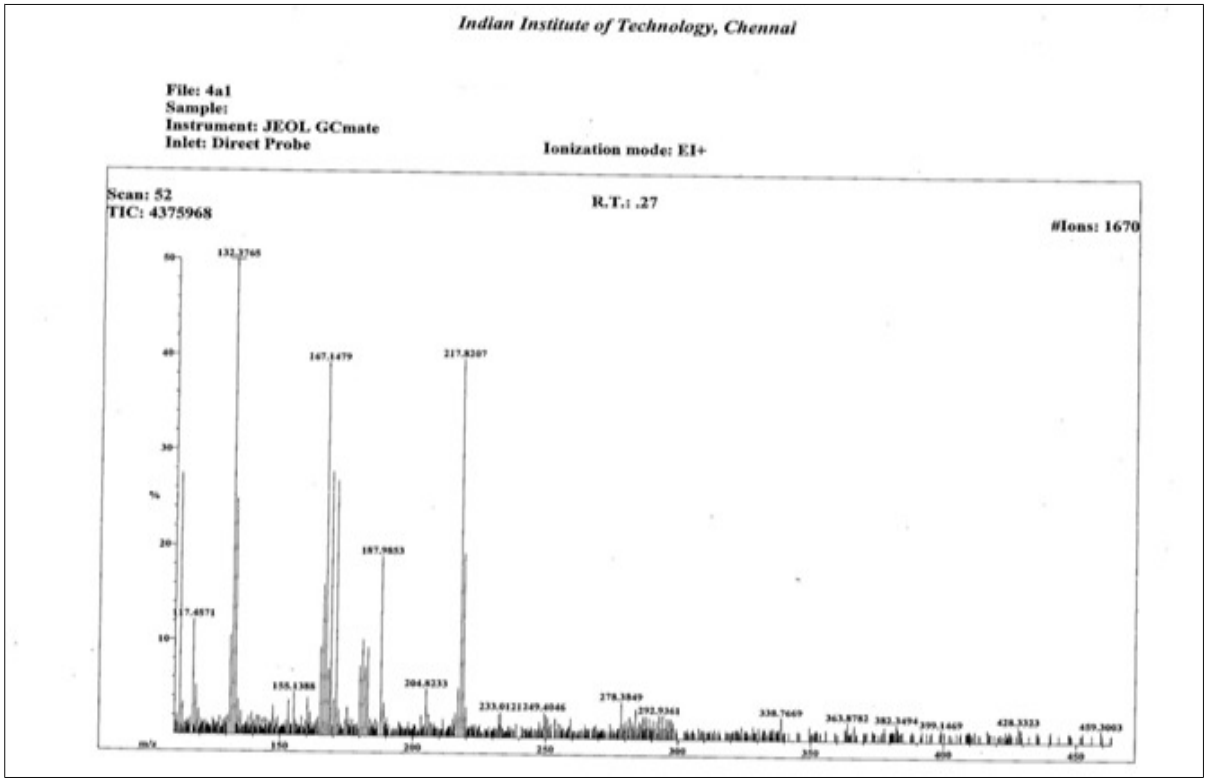

Figure 20: Mass Spectrum of he Compound $4a_1$

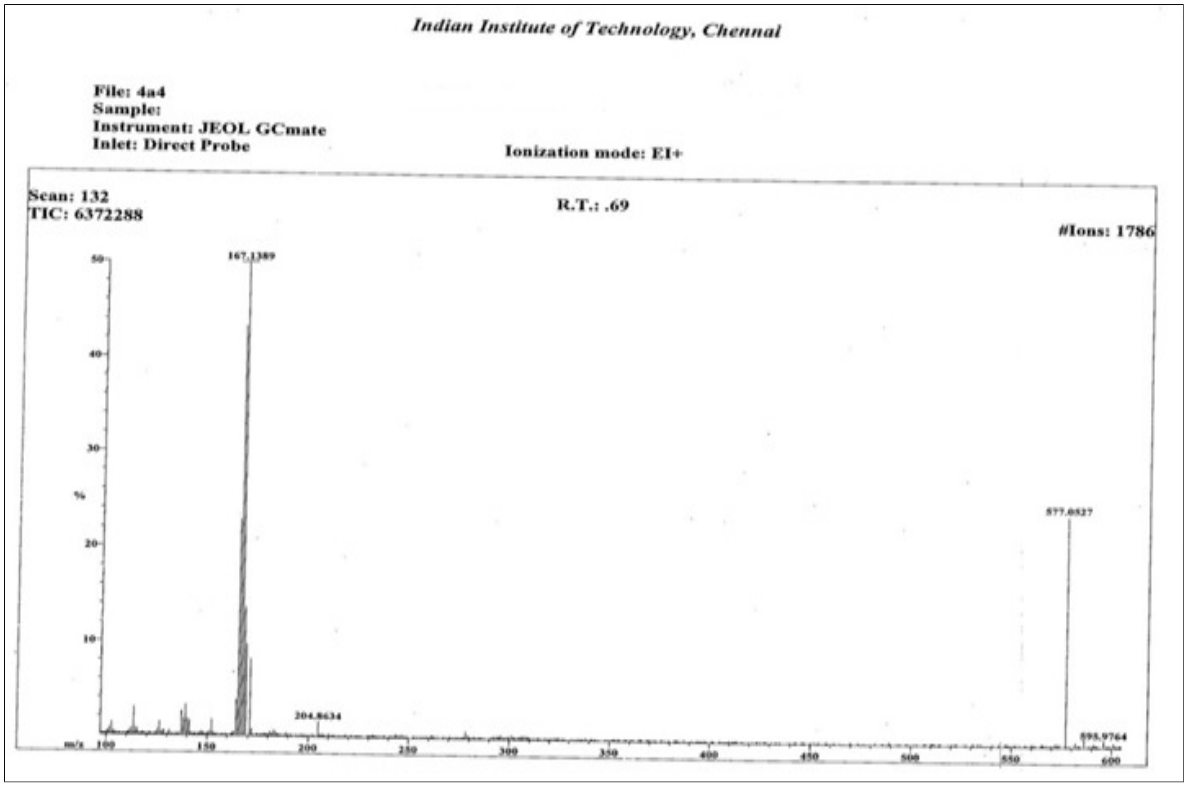

Figure 21: Mass Spectrum of the Compound $4a_4$

Table 13: NMR Spectral Data of Synthesized Compounds

COMPOUND	1H NMR ($CDCL_3$-d, δppm)
$4a_1$ 2(d, 1H, $-NH_2$), 4.62 (S, 4H, $-CH_2$)7.55-7.92 (M, 4H,	

Ar-CH), 7.2 (S, 1H, -NH).	
$4a_2$	2.35(S, 3H, -CH_3), 4.62 (S, 2H,-CH_2), 7.0(S, 1H, -NH), 7.3-7.98(M, 9H, Ar-H).
$4a_3$	4.62(S, 2H, CH_2), 5(S, 1H, -OH), 6.8-7.98(M, 8H, Ar-H), 8.0(S, 1H, -NH), 8.1(S, 1H, CH).
$4a_4$	4.82(S, 2H, CH_2), 6.8-8.1 (M, 8H, Ar-H), 8.7(S, 1H, -NH), 8.2(S, 1H, CH), 2.35(S, 6H, CH_3).

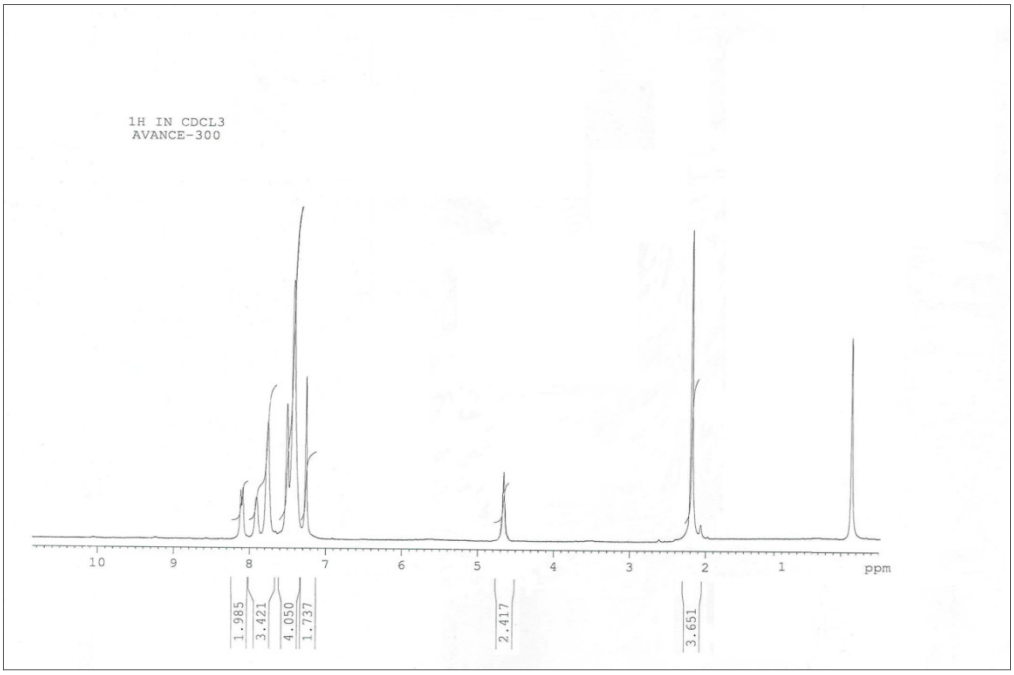

Figure 22: 1H NMR Spectrum of the Compound $4a_1$

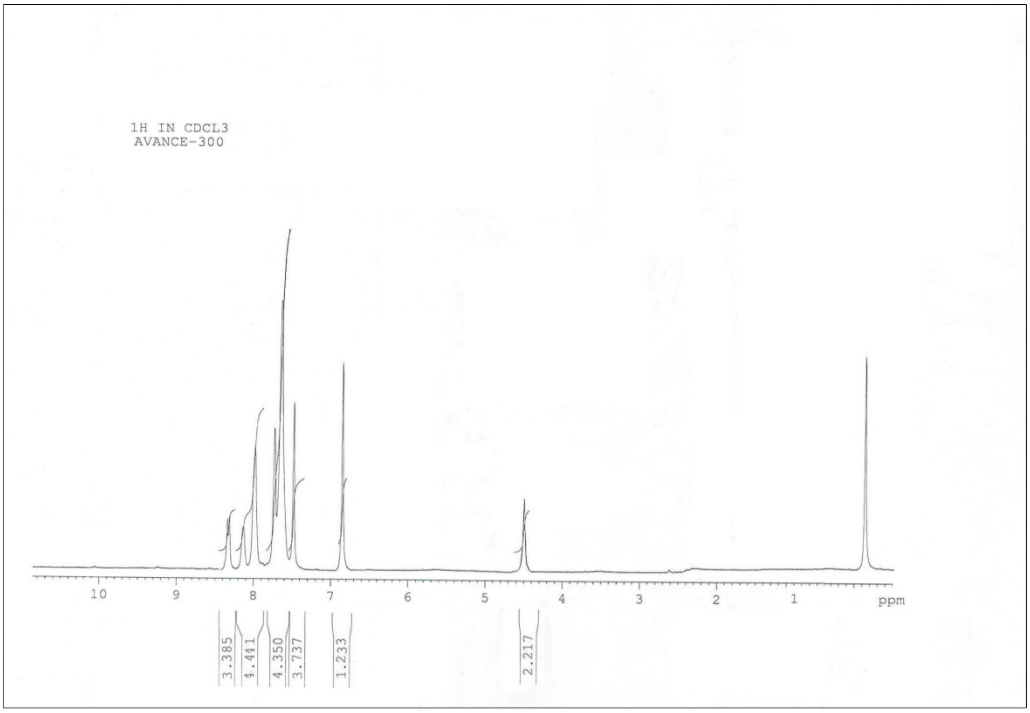

Figure 23: 1H NMR Spectrum of the Compound $4a_2$

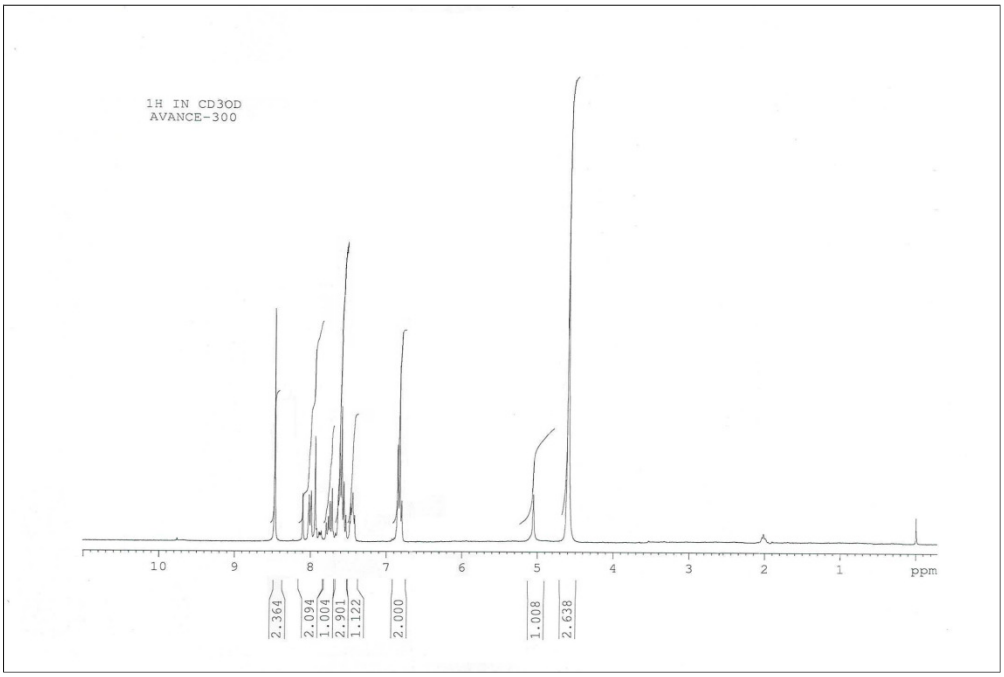

Figure 24: 1H NMR Spectrum of the Compound $4a_3$

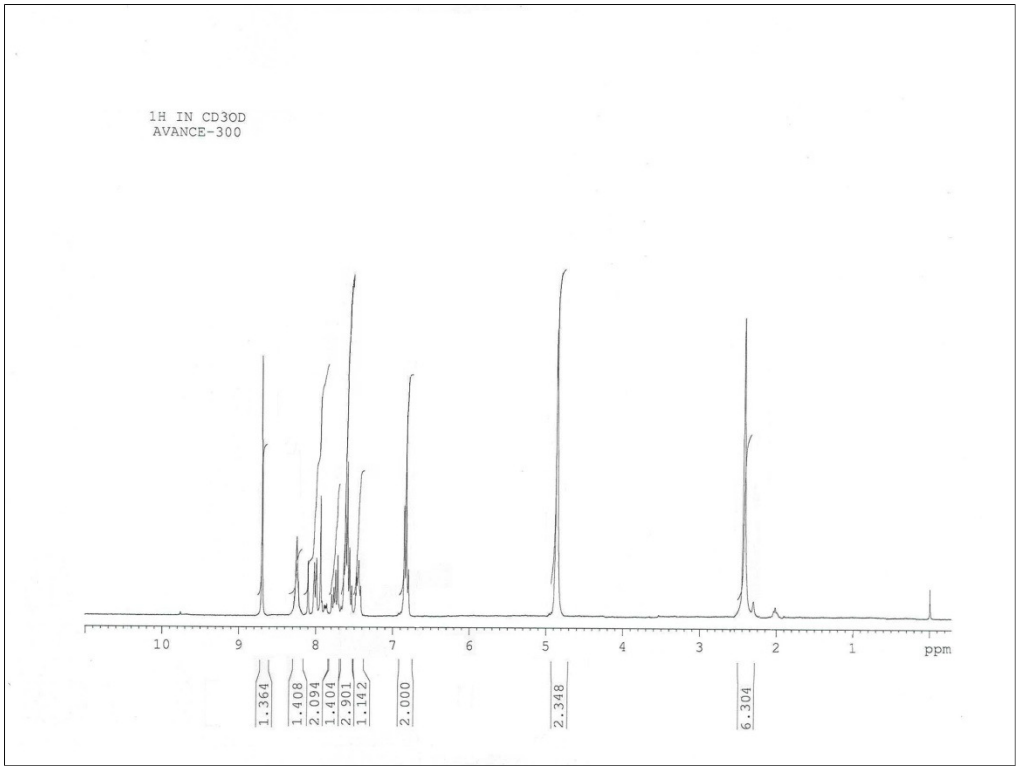

Figure 25: 1H NMR Spectrum of the Compound $4a_4$

6.1.5. Study of In vitro Hepatoprotective Activity.

The resulting liver dysfunction as marked by the increase of SGPT, SGOT and ALP activities. $4a_1$ and $4a_4$ decreased the alcohol-induced increase of SGPT, SGOT and ALP activities dose dependently. However, 50 mg/kg bodyweight dose of $4a_4$ was found to be most effective. This dose was therefore selected for next experiment on time-dependent effect of alcohol. Treatment of alcohol for different time periods (0-35 days) showed a linear increase in the activities of the liver marker enzymes, SGPT, SGOT and ALP till 12 days and at 15th day there was no significant additional effect over day 15.$4a_1$ significantly decreased alcohol-induced increase of enzyme activities, suggesting its hepatoprotective effect.

UDP-glucuronosyl transferase (UGT) activity is known to prevent the generation of cellular oxidative stress by glucuronidation and subsequent exclusion of harmful metabolites. UGT therefore plays an important role in the rescue of cellular damage due to stress. This prompted us to investigate UGT in some detail in the liver. Since 50 mg/kg body weight dose

of $4a_1$ was found to be most effective in protecting liver damage, this dose was selected for UGT-related studies. Alcohol treatment affected microsomal UGT activity that was evident from the reduction of PNP-glucuronidation and $4a_1$ prevented the deterioration of glucuronidation activity effectively. It could be seen from that alcohol treatment caused a significant decrease ($p < 0.001$) in UGT gene expression while co-treatment with $4a_1$ protected the down regulation of this gene expression.

Table 14: Effect of dosage on ALP activity

S.No	Drug	Optical Density

1	Control	18 ± 0.9
2	Ethanol	59 ± 6.3
3	Ethanol+4a_4 -2.5	42 ± 7.3
4	Ethanol+4a_4-5.0	27 ± 1.9
5	Ethanol+4a_4-10.0	29 ± 1.7

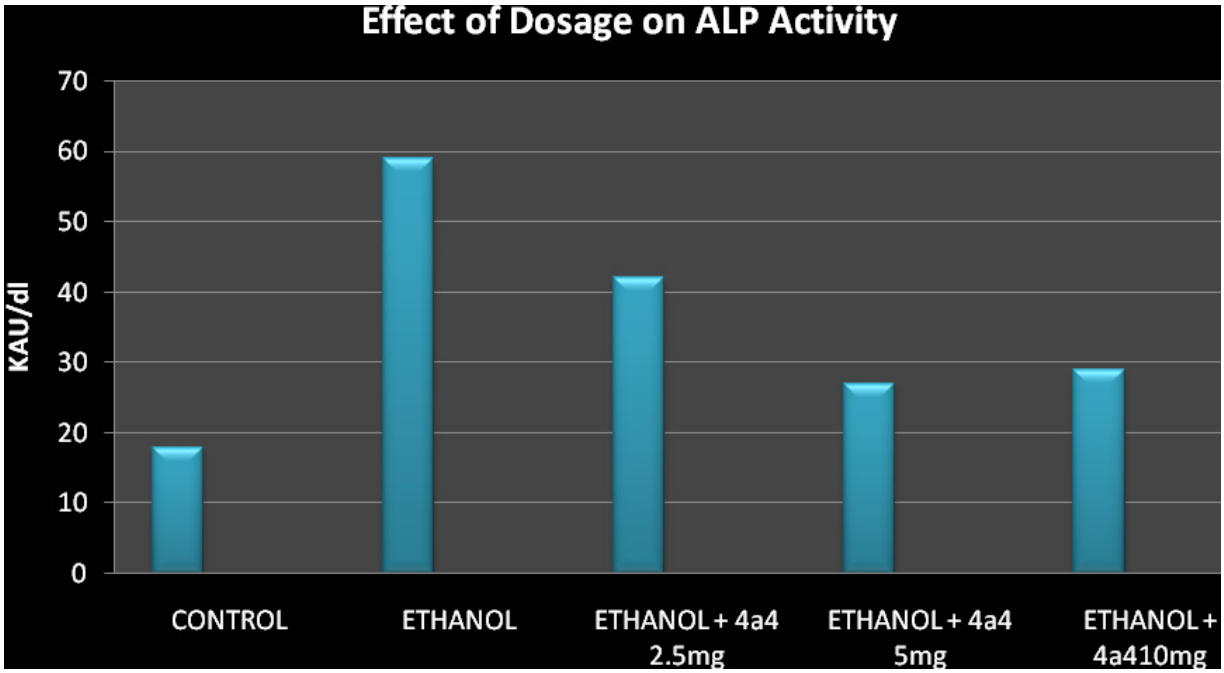

Figure 26: Effect of Dosage on ALP activity

Table 15: Effect of dosage on SGOT activity

S.No	Drug	Optical Density

1	Control	45 ± 4.2
2	Ethanol	135 ± 10.5
3	Ethanol+4a_4-2.5	127 ± 11.83
4	Ethanol+4a_4-5.0	65 ± 5.2
5	Ethanol+4a_4-10.0	68 ± 4.8

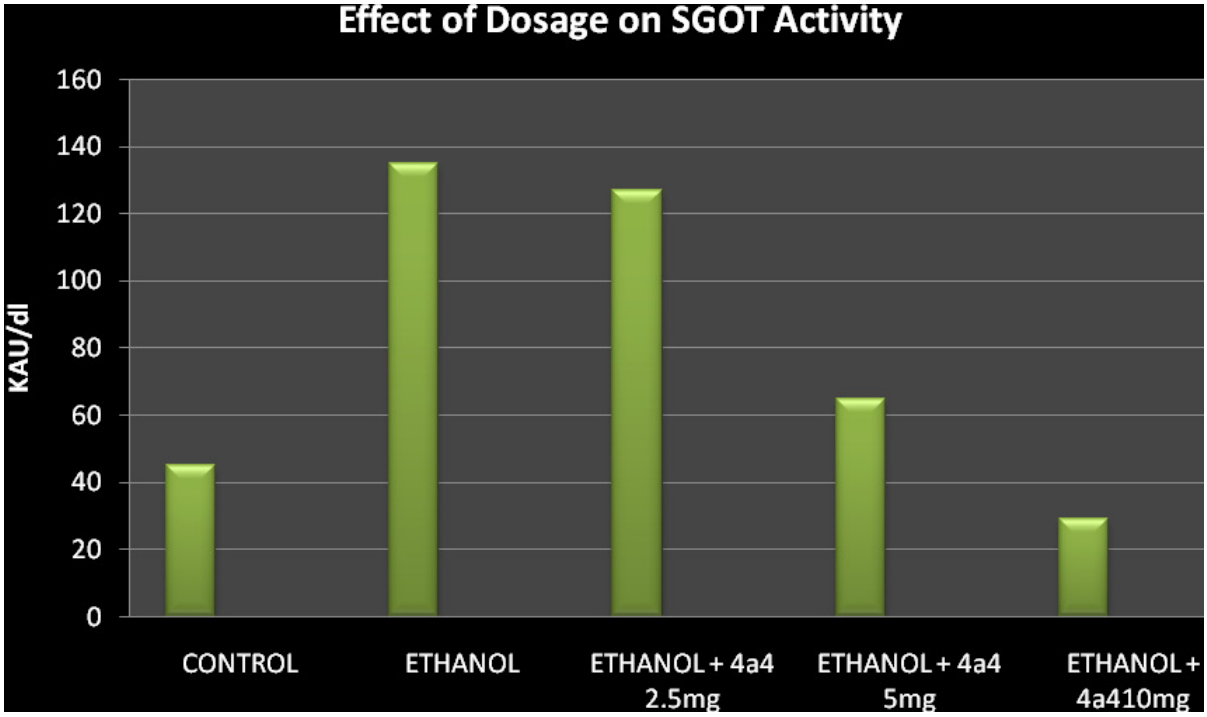

Figure 27: Effect of Dosage on SGOT activity

Table 16: Effect of dosage on SGPT activity

S.No	Drug	Optical Density
1	Control	22 ± 2.0
2	Ethanol	196 ± 15.3
3	Ethanol+4a_4-2.5	136 ± 9.54
4	Ethanol+4a_4-5.0	72 ± 4.6
5	Ethanol+4a_4-10.0	81 ± 8.7

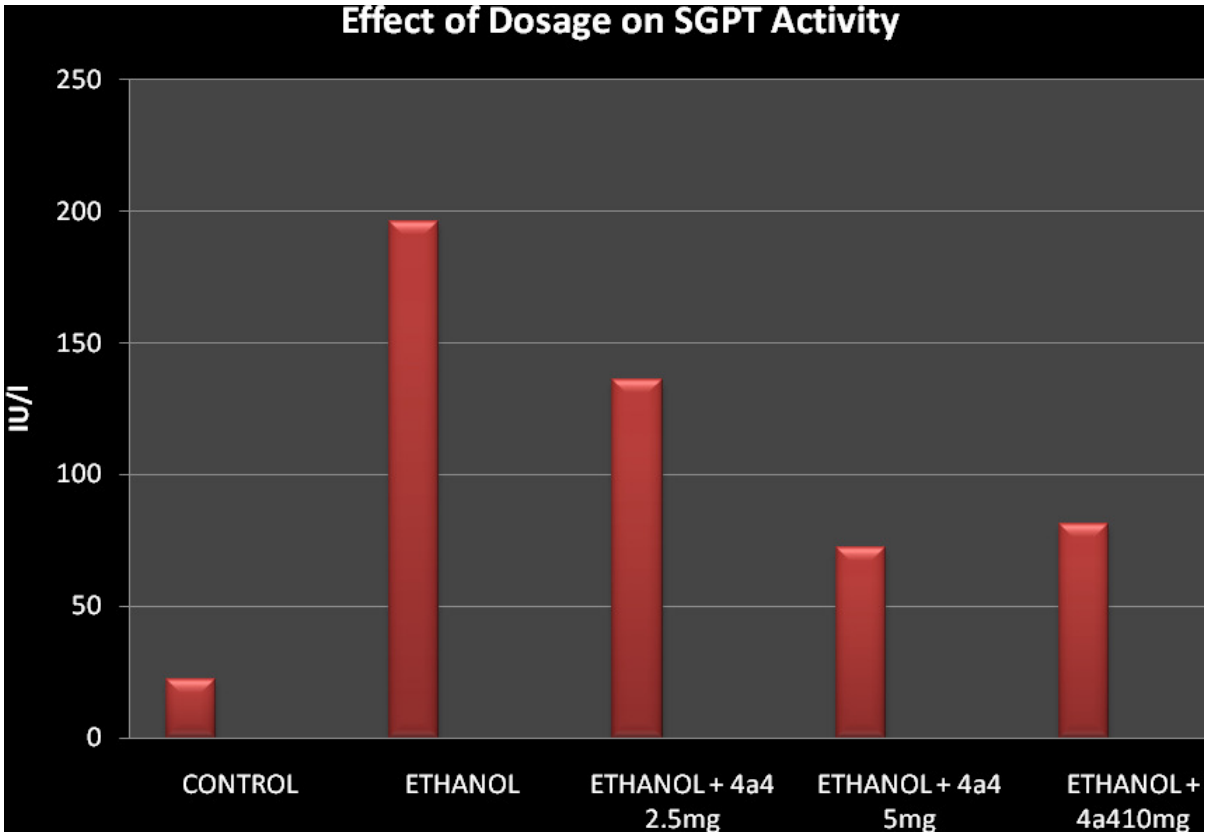

Figure 28: Effect of Dosage on SGPT activity

Table 17: Measurement of PNP–UGT activity against synthesized compound $4a_1$

S.No	Drug	PNP-UGT activity of non activated microsomes	PNP-UGT activity of activated microsomes
1	Control	61 ± 5.2	23 ± 1.1
2	Ethanol	40 ± 3.5	17 ± 0.9
3	Eth+$4a_1$	67 ± 4.7	29 ± 1.2

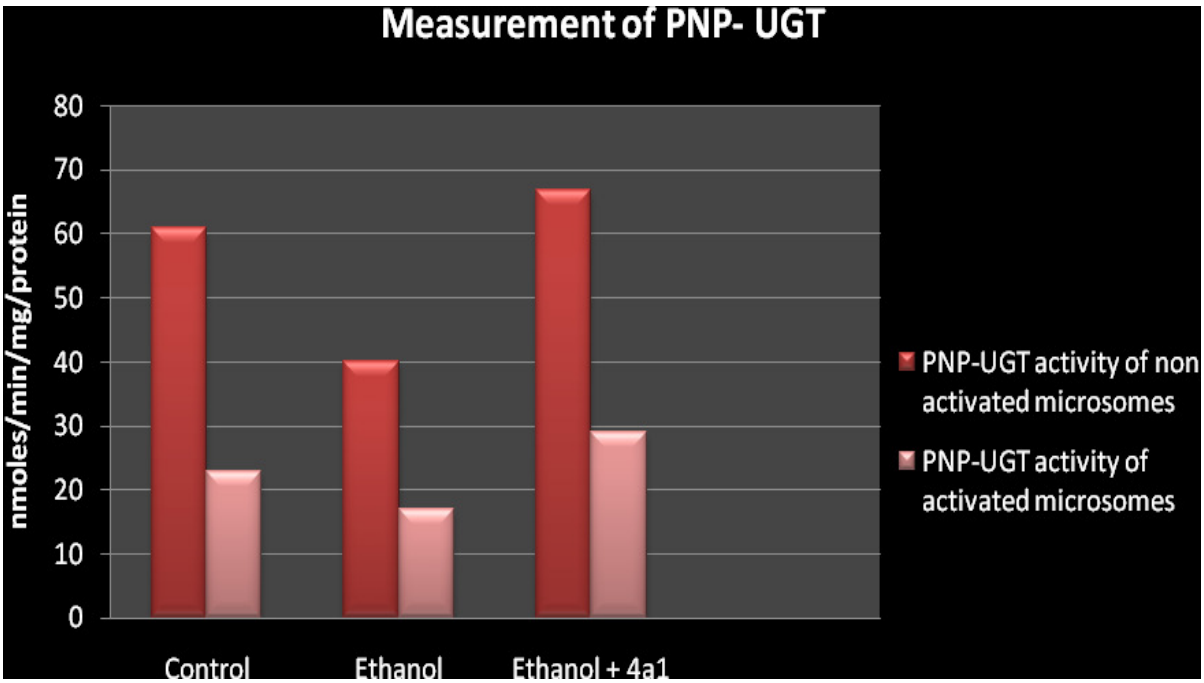

Figure 29: Measurement of PNP–UGT activity against synthesized compound $4a_1$

Table 18: Treatment of alcohol extract for different time periods (0–35 days)

S.No	Days	0	7	14	21	28	35
1	Control	51	48	43	45	51	48
2	Ethanol	51	47	58	76	68	69
3	Ethanol+ $4a_1$	51	66	71	101	143	141

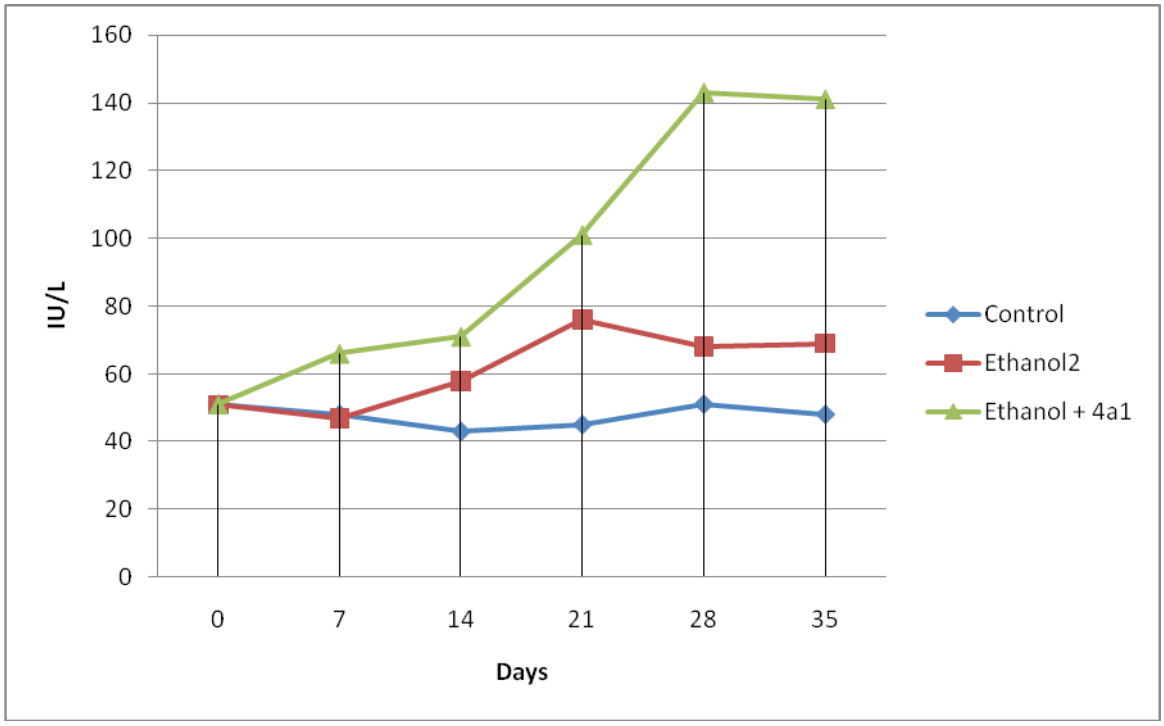

Figure 30: Treatment of alcohol extract for different time periods (0–35 days)